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SOCIETY OF AMERICAN BACTERIOLOGISTS

MONOGRAPHS ON SYSTEMATIC BACTERIOLOGY

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MONOGRAPHS ON SYSTEMATIC BACTERIOLOGY

A COMPILATION OF CULTURE MEDIA

FOR THE CULTIVATION OF MICROORGANISMS

BY

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FOREWORD

The contributions relating to the various substrates and media used for the cultivation of bacteria, yeasts, and molds, are widely scattered in the literature. Very frequently they are not adequately indexed. For some years it has been felt that a key to the various media and substrates proposed for the growth of microörganisms would prove helpful. The fact that these formulae were not readily available has led to a great amount of duplication in the literature. A search of the literature has therefore been undertaken and the formulae for about 7000 various media that have been proposed brought together. The task of securing this material was performed most acceptably by Mr. H. W. Schoenlein, who was also associated in the development of the keys The task of organizing the compilation, of planning the presentation, and of final editing was undertaken by Dr. Max Levine. It was found possible greatly to curtail the number of separate formulae by use of the subheading Variants in many cases.

The compilation presented in the following pages was made possible by a grant to the Society of American Bacteriologists by the Digestive Ferments Company of Detroit, Michigan. At the request of the Society of American Bacteriologists, the Department of Bacteriology at the Iowa State College accepted the task of compilation. The interest, helpfulness, and support of the Digestive Ferments Company throughout the progress of the work cannot be too greatly appreciated.

The system of classification of the media, together with the arrangement of keys, indices, etc., should make possible a ready review of the media which have been proposed for growing different types of organisms and containing different ingredients. The primary subdivisions into Groups on the basis of physical characteristics and nature of the solidifying agent is the logical one both from the standpoint of history and the standpoint of usefulness. The secondary subdivisions based upon the chemical characteristics of the constituents of the media are in line with modern trend toward emphasis upon physiology and metabolism of microörganisms.

It is hoped that this compendium of information will prove very generally useful to all bacteriologists and in all bacteriological laboratories.

R. E. Buchanan.

PREFACE

The rapid growth of the science of Bacteriology has led to the development of numerous combinations of materials for the propogation of microörganisms. It was felt that the collection and orderly arrangement of such culture media would be of assistance in the future development of the science of Bacteriology, and the authors were gratified with the opportunity to perform this service.

In spite of the efforts to thoroughly cover the literature, this compilation is probably incomplete, particularly with respect to formulae published in bulletins and monographs which were not available or consulted. It is hoped that this compilation will help acquaint the bacteriologists with the numerous types and combinations of materials which have been employed for cultivating microorganisms, and if it serves to check the needless publication of new formulae, which are really old, the authors will feel well repaid for their time and efforts.

To Mr. H. G. Dunham, of the Digestive Ferments Company, we are greatly indebted for helpful criticisms, and suggestions.

To Mrs. C. H. Werkman, Miss Ruth Confare and Miss Lois Kratoska, the authors extend cordial thanks for assistance in typewriting and correcting the manuscript.

To Dr. R. E. Buchanan the authors wish to particularly express their indebtedness and gratitude for the fatherly advice, encouragement, and assistance, so graciously rendered at all times.

MAX LEVINE,
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Ames, Iowa,
July 1, 1929

INTRODUCTION

At the request of the Society of American Bacteriologists, the literature was surveyed for formulae of culture media reported useful for the growth of bacteria and other microörganisms. The standard periodicals such as the various Centralblatts für Bakteriology, Zeitschrift für Hygiene, Annals of the Pasteur Institute, Journal of Hygiene, Journal of Pathology and Bacteriology, and all of the American bacteriological journals were perused from their inception to the year 1926. A number of other periodicals, as well as text books, monographs and many Experiment Station bulletins were included in the survey.

Formulae of about seven thousand culture media were gleaned from the literature. These were arranged and classified into approximately twenty five hundred quite distinct media. A number and a distinctive name was assigned to each medium which is described under the following headings:—Constituents, Preparation, Sterilization, Use, Variants, so as to facilitate comparison of different formulae.

A **dichotomous key,** several indices and complete references to all articles from which formulae were taken are also included in this compilation. In the key, and all indices, the numbers refer to *medium numbers* and not pages.

The **Medium Name Index** includes an alphabetical list of all media described herein.

In the **Constituents Index** an attempt was made to list every medium in which a particular substance was employed.

The **Author Index** is self explanatory.

The Use Index is necessarily incomplete. It includes only those media for which a specific use was indicated in the original article reviewed.

The various media were subdivided into seven "groups" on the basis of their physical states. Thus, all the liquid media are considered in a group by themselves and the solid media are further subdivided, on the nature of the solidifying agent and physical properties of these agents, as, for example, whether initially liquid, reversibly liquid and solid, or permanently solid.

Each group, if sufficiently complicated to warrant further classification, is then subdivided into "sub-groups" on the basis of the presence or absence of additional organic constituents.

The sub-groups are further subdivided into sections on the basis of the nature of the nitrogen, carbon, and inorganic constituents.

For each section there is prepared a key, generally dichotomous, by means of which each medium therein may be located. The various keys are distributed throughout the book immediately preceding their respective media.

The problem of a suitable means of identification of individual media was a particularly difficult one. The scheme finally adopted was to assign a number in conjunction with a specific name, the latter including the chief nitrogen and carbon source, where possible, and the name of the author who first listed the medium. Thus "273 Ayers' Glucose Ammonium Phosphate Solution," indicates that Ayers first described the medium, the nitrogen source was ammonia, the carbon source glucose, and that the medium is No. 273 in this collection.

In addition to the carbon and nitrogen source the nature of the solidifying agent is also employed in naming culture media, as, for example, "1553 Greig Smith's Sucrose Peptone Agar."

Where the original article containing a medium was not available the fact is indicated by placing the author from whose publication the medium was obtained in parentheses after the name of the medium. Thus "2156 Beck's Glycerol Serum Agar (Klimmer)" indicates that the medium was obtained from Klimmer who referred to Beck as the author.

If the same medium is described by different authors, only the earliest available author's name appears in the medium name, but the other authors are given under the heading **"references"** at the end of the description of the medium.

Where several media differ only with respect to the relative quantities of constituents employed, or in the method of preparation, the earliest described medium takes precedence, and all others are described as **variants.**

One of the vexing questions which arose was that of the disposition of media in which it was specified that a number of substances might be substituted for each other. For example, if the availability of various carbon sources is being studied, shall each new combination, as the employment of glucose, sucrose, glycerol, etc., be considered a distinct medium? To do so would have markedly extended the number of media to be considered.

The term "basal" was introduced to take care of this situation. This was applied to the formula exclusive of the substituted constituents which then became **added nutrients.** The term "basal" occurring in the name of a medium therefore designates that the formula is not in iteslf a complete medium, but that it serves as a base to which other constituents are added.

A serious difficulty in naming media arose in connection with the use of beef extracts and meat infusions. This was particularly troublesome when authors did not specify whether extract or infusions were employed. Where the use of extracts was specified in the articles reviewed, these terms appear in the names of the respective media, as e.g., "779 Dunham's Infusion Broth" or "1695 Heinemann's Meat Extract Agar." Where the author did not specify the nature of the material employed (i.e., whether extract or infusion) the term "bouillon" is employed, thus "936 Kendall, Day and Walker's Mannitol Bouillon."

COMPOSITION OF CULTURAL MEDIA AND SUBSTRATES USED IN BACTERIOLOGY

Any classification of media and substrates used in bacteriology must of necessity be artificial and arbitrary in form. In the classification here adopted an effort has been made to bring closely related media together, although it is realized that in some cases even the primary divisions tend to separate media having much the same essential composition and used for much the same purposes.

The 7 primary divisions which have been adopted are as follows:

PRIMARY CLASSIFICATION OF MEDIA

A_1. Liquid media.. Group I (Med. 1–1394)
A_2. Solid media.
    B_1. Initially liquid.
        C_1. Reversible. Reversibly liquid and solid. Liquefiable by heat.
            D_1. Solidified by the addition of agar-agar.. Group II (Med. 1395–2198)
            D_2. Solidified by the addition of gelatin.. Group III (Med. 2199–2371)
            D_3. Solidified by the addition of other materials Group IV (Med. 2372–2382)
        C_2. Irreversible. Not reversibly liquid and solid.
            D_1. Solidifying agent organic.............. Group V (Med. 2383–2466)
            D_2. Solidifying agent inorganic........... Group VI (Med. 2467–2485)
    B_2. Initially solid.................................. Group VII (Med. 2486–2543)

GROUP I. LIQUID MEDIA

The term "Liquid Medium" is here defined to include all media containing sufficient water and so lacking in viscosity as to be readily poured from one vessel to another. A liquid medium is considered one which does not "set" or solidify when cooled to any temperature above the freezing point of water.

The classification of liquid media adopted is based primarily upon whether the nitrogen supplied is inorganic or organic.

Subgroups of liquid media

A_1. Water only..............Subgroup I-A
A_2. Water with other constituents
    B_1. All constituents of medium inorganic Subgroup I-B (Med. 2 to 113)
    B_2. One or more constituents organic Subgroup I-C (Med. 114 to 1394)

SUBGROUP I-A

The water used in the bacteriological laboratory as a medium or in the preparation of media may be either distilled or a natural water. In theory distilled water is to be preferred, although distilled water unless carefully prepared may contain gaseous or metallic impurities which will interfere with its use.

Distilled water for the bacteriological laboratory.

Surface and ground water for media.

1. Molisch's Basal Nutrient Solution

Constituents:

1. Sea water..................1000.0 cc.

Preparation:
(1) Dissolve one of the added nutrients in amount indicated in 1.
(2) Tube in tall tubes. Fill within a centimeter of the plug.

Sterilization: Method not given.

Use: To determine the constituents essential for the growth of purpurbacteria, *Rhodobacterium capsulatum* and *Rhodobacillus palustris* (Molisch). The cultures were incubated in diffused light. The author reported generally no growth using sucrose, dextrin, glycerol, ammonium tartrate or asparagin alone, but generally growth with the other materials and combinations listed.

Added nutrients and variants: The author suggested the following:
(a) Add one of the following materials:
sucrose 1.0%
dextrin 1.0%
inulin 1.0%
asparagin 1.0%
peptone 1.0%
glycerol 1.0%
peptone 1.0% + sucrose 0.5%
peptone 1.0% + glycerol 0.5%
peptone 1.0% + dextrin 0.5%
peptone 1.0% + asparagin 0.5%
peptone 1.0% + inulin 0.5%
asparagin 0.5% + dextrin 0.5%
asparagin 0.5% + inulin 0.5%
ammonium tartrate 1.0%
(b) Used Moldau river water added one of the following materials:
sucrose 1.0% or 0.5%
dextrin 1.0% or 0.5%
inulin 1.0% or 0.5%
asparagin 1.0% or 0.5%
glycerol 1.0% or 0.5%
peptone 1.0%
peptone 1..% + sucrose 1.0% or 0.5%
peptone 1.0% + dextrin 1.0% or 0.5%
peptone 1.0% + inulin 1.0% or 0.5%
peptone 1.0% + asparagin 1.0% or 0.5%
peptone 1.0% + glycerol 1.0% or 0.5%
asparagin 0.5% + dextrin 0.5%
asparagin 0.5% + glycerol 0.5 g.
dextrin 0.5% + inulin 0.5%
ammonium tartrate 1.0%
sodium citrate (neutral) 0.05%
ammonium citrate 0.05%
magnesium citrate 0.05%
potassium citrate 0.05%
calcium butyrate 0.05%
sodium acetate 0.05%
iron ammonium citrate 0.05%
iron magnesium citrate 0.05%
iron ammonium citrate 0.05% + gypsum
infused hay
infused hay + iron ammonium citrate 0.05%
iron potassium citrate 0.05%
iron ammonium tartrate 0.05%
iron ammonium oxalate 0.05%
iron glycero phosphate 0.05%
iron lactate 0.05%
iron magnesium lactate 0.05%
manganese peptone 0.025%
manganese peptone 0.025% + K_2HPO_4 0.1%
manganese peptone 0.025% + K_2HPO_4 0.1% + KNO_3 0.1% + $MgSO_4$ 0.1%
manganese peptone 0.025% + K_2HPO_4 0.1% + KNO_3 0.1%
manganese peptone 0.025% + $MgSO_4$ 0.1%
manganese peptone 0.025% + KNO_3 0.1%
manganese peptone 0.025% + gypsum 0.1%
manganese peptone 0.025% + $K_4Fe(CN)_6$ 0.1%
manganese peptone 0.025% + citrate iron peptone
peptone 0.5%
peptone + $MnCO_3$ in excess
peptone + $FeCO_3$ in excess
iron albuminate 0.05%
iron peptonate 0.05%
manganese lactate 0.05%
manganese phospholactate 0.05%
manganese salicylicate 0.05%
manganese fluorate 0.05% + peptone 0.5%
manganese citrate 0.05%
manganese oxalate 0.05%
manganese glycerinate 0.05%
iron sulphide—peptone 0.05%
manganese acetate 0.05%
(c) Sea water without any addition.
(d) Moldau river water without any addition.

Reference: Molisch (1907, p. 68).

SUBGROUP I-B

Liquid Media in which All Constituents are Inorganic

Liquid media in which all constituents are inorganic may be classified most conveniently on the basis of the type of nitrogen provided.

Key to the sections of Subgroup I-B

A_1. Nitrogen present as free or elementary nitrogen only.
  B_1. Incomplete media, so-called "basal" solutions, requiring the addition of other nutrients..........Section 1 (Med. 2–25)
  B_2. Complete solutions, used as media without additions.....Section 2 (Med. 26–29)
A_2. Nitrogen supplied as ammonium salts.
  B_1. Incomplete media, or basal solutions. Section 3 (Med. 30–56)
  B_2. Complete media.
    C_1. Media primarily for organisms oxidizing ammonia to nitrites for growth energy...........Section 4 (Med. 57–72)
    C_2. Media not primarily for organisms oxidizing ammonia to nitrites for growth energy...........Section 5 (Med. 73–85)
A_3. Nitrogen supplied as nitrites.
  Media primarily for organisms oxidizing nitrites to nitrates for growth energy. Section 6 (Med. 86–95)
A_4. Nitrogen supplied as nitrates.
  B_1. Incomplete or basal solutions requiring the addition of other nutrients. Section 7 (Med. 96–106)
  B_2. Complete nutrient solutions. Section 8 (Med. 107–113)

SUBGROUP I-B. SECTION 1

Inorganic basal solutions of known chemical composition, nitrogen present only as atmospheric nitrogen; incomplete solutions requiring the addition of other nutrients.

A_1. Containing salts of monovalent cations only.
  Physiological Salt Solution. (Normal saline)............................ 2
  Molar Salt Solution.................. 3
A_2. Containing salts of monovalent and other cations.
  B_1. Salts of mono and divalent cations present.
    C_1. Sodium salts present.
      D_1. Calcium salts present.
        Ashby's Basal Solution. (Hoffmann and Hammer)........................ 4
        von Wahl's Basal Salt Solution....... 5
        Locke's Solution..................... 6
        Winogradsky's Basal Inorganic Salt Solution (Heinemann)............. 7
        Charrin and Dissard's Basal Salt Solution................................ 8
        Gerlach and Vogel's Basal Solution................................ 9
        Rettger, Berman and Sturges' Basal Solution.......................... 10
      D_2. Calcium salts not present.
        Omeliansky's Basal Salt Solution..... 11
        Percival's Basal Salt Solution........ 12
        Nawiasky's Basal Salt Solution....... 13
    C_2. Sodium salts not present.
      Stoklasa's Basal Solution............. 14
      Heinze's Basal Salt Solution.......... 15
      Söhngen's Basal Solution............. 16
      van Delden's Basal Gypsum Solution................................ 17
      Czapek's Basal Solution... 18
      Buchanan's Basal Salt Solution 19
  B_2. Salts of mono, di, and trivalent cations present.
    C_1. Sodium Salts present.
      Fuhrmann's Basal Solution........... 20
      Münter's Basal Salt Solution......... 21
      Meyer's Basal Salt Solution. (Perotti)............................. 22
    C_2. Sodium Salts not present.
      Dox's Inorganic Salt Solution for Fungi (Tanner).................... 23
      Gage's Basal Salt Solution............ 24
      Bijerinck and van Delden's Basal Salt Solution.......................... 25

2. Physiological Salt Solution (Normal saline)

Constituents:

1. Water	1000.0 cc.
2. NaCl (c.p.)	8.5 g.

Preparation: (1) Dissolve 2 in 1.

Sterilization: As desired.

Uses: Diluent and serological work in the laboratory.

Variants: Frost used 6.0 g. NaCl per liter

References: Original author not found. This formula was taken from Giltner (1921, p. 399). Frost (1903, p. 18).

3. Molar Salt Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl (best commercial grade)	60.0 g.

Preparation: (1) Dissolve 2 in 1.

Sterilization: As desired.

Uses: Diluent.

References: Giltner (1921, p. 399).

4. Ashby's Basal Solution (Hoffmann and Hammer)

Constituents:

1. Distilled Water	1000.0 cc.
2. KH_2PO_4	0.2 g.
3. $MgSO_4$	0.2 g.
4. NaCl	0.2 g.
5. $CaSO_4$	0.1 g.
6. $CaCO_3$	5.0 g.

Preparation:

(1) Dissolve the KH_2PO_4 in a small amount of water, and add tenth normal NaOH to neutral point to phenolphthalein.

(2) Dissolve 3, 4, 5 and 6 in remainder of 1

(3) Mix (1) and (2).

(4) Add suitable carbon source.

(5) Distribute in 20.0 cc. lots in 150 cc. Erlenmeyer flasks.

Sterilization: Method not specified.

Uses: For study of the nitrogen fixing bacteria from soil.

Added Nutrients: Hoffmann and Hammer suggest the following list of carbon sources:

Mannitol	20 g.
Maltose	20 g.
Sucrose	20 g.
Galactose	20 g.
Inulin	20 g.
Dextrose	20 g.
Levulose	20 g.
Dextrin	20 g.
Raffinose	20 g.
Lactose	20 g.

References: Hoffmann and Hammer (1910 p. 128).

5. von Wahl's Basal Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.3 g.
5. NaCl	0.1 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add one of the added nutrients, or combinations of added nutrients.

Sterilization: Not specified.

Use: Cultivation of carrot bacillus (*Bacillus daucarum*) and other organisms causing vegetable spoilage.

Added nutrients: The author added one of the following materials or combinations of materials:

(a)	peptone	10.0 g.
(b)	sucrose	30.0 g.
	asparagin	10.0 g.
	glycerol	10.0 g.
(c)	sucrose	30.0 g.
	asparagin	1.0 g.
(d)	glycerol	30.0 g.
	asparagin	1.0 or 10.0 g.
(e)	lactose	30.0 g.
	asparagin	10.0 g.
(f)	sucrose	5.0 g.
	glycerol	10.0 g.
	ammonium tartrate	10.0 g.
(g)	sucrose	5.0 g.
	glycerol	10.0 g.
	KNO_3	10.0 g.
(h)	KNO_2	0.5 g.
	soda	0.5 g.
(i)	sucrose	5.0 g.
	glucose	5.0 g.
	glycerol	5.0 g.
(j)	glucose	30.0 g.
	asparagin	10.0 g.
(k)	$(NH_4)_2SO_4$	0.0025 g.
	Na_2CO_3	5.0 g.

Reference: von Wahl (1906, p. 496).

6. Locke's Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl (0.9 to 1.0%)	9.0 to 10.0 g.
3. KCl (0.01%)	0.1 g.
4. $CaCl_2$ (0.02%)	0.2 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: As desired.

Uses: Commonly used as a diluent. Tissue neither swells nor shrinks in this solution. It is also used as a basis for culture media, various nitrogen or carbon sources being added.

Reference: Park, Williams and Krumwiede (1924, p. 122).

7. Winogradsky's Basal Inorganic Salt Solution (Heinemann)

Constituents:

1. Distilled Water	1000.0 cc.
2. $MgSO_4$	0.5 g.
3. $CaCl_2$	0.01 g.
4. NaCl	2.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1

Sterilization: Not specified.

Uses: Used in soil bacteriological work as a base to which other materials are added.

Reference: Heinemann (1922, p. 37).

8. Charrin and Dissard's Basal Salt Solution

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4	0.1 g.
3. Na_2HPO_4	0.1 g.
4. $CaCl_2$	0.05 g.
5. $MgSO_4$	0.05 g.
6. $KHCO_3$	0.134 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Adjustment of reaction not given.

(3) Distribute in 50.0 cc. lots in flasks and add 1.0 or 0.5 g. of one of the added nutrients to each flask.

Sterilization: Autoclave at 120°C. Time not specified.

Use: To study pigment production by *Bacillus pyocyaneus.* The authors reported that asparagin gave the best pigment; peptone gave a little pigment; no growth using urea.

Added nutrients: The authors added 0.5 or 1.0 g. of one of the following organic materials to each 50.0 cc. lot of medium:

peptone
asparagin
urea
glucose
glycogen
acetic acid
lactic acid

Reference: Charrin and Dissard (1893, p. 182).

9. Gerlach and Vogel's Basal Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	0.5 g.
3. $CaCO_3$	0.5 g.
4. NaCl	0.5 g.
5. $FeSO_4$	some

Preparation:

(1) Dissolve 2, 3, 4, 5 and one of the carbon sources listed under added nutrients in 1.

(2) Distribute in flat bottomed flasks.

Sterilization: Steam for 30 minutes on each of 3 successive days.

Use: Study of nitrogen assimilation by azotobacter and other bacteria from the soil. More nitrogen was assimilated using dextrose as a source of carbon than calcium propionate. Little nitrogen was assimilated if the flasks were sealed after inoculation.

Added nutrients:

(a) The authors suggested the use of the following carbon sources:

Glucose	2.0 g.
Calcium propionate	1.0 g.

(b) Percival used the same basic solution and added 2.0 g. glucose.

Variants: Bonazzi added 10.0 g. of glucose, designated the use of deep well water added 1.0 g. $CaCO_3$, specified the addition of 0.02 to 0.5 g. $FeSO_4 \cdot 7H_2O$, and also stated that 0.23 g. KNO_3 and 1.264 g. $Ca(NO_3)_2$ might be added if desired.

References: Gerlach and Vogel (1902, p. 671), Percival (1920, p. 181), Bonazzi (1921, p. 339).

10. Rettger, Berman and Sturges' Basal Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl	5.0 g.
3. Na_2SO_4	2.0 g.
4. $CaCl_2$	1.0 g.
5. KH_2PO_4	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add one of the added nutrients listed below.

Sterilization: Generally by filtering through a Berkefeld filter, in some cases by heat.

Use: To study proteolysis by *Proteus vulgaris, B. prodigiosus* and *B. subtilis.*

Added nutrients:

(a) Various chemically pure proteins added alone or in combination.

(b) Egg albumin prepared by method of Hopkins and Pinkus (1899); amount not specified.

(c) Peptone 10.0 g.

Reference: Rettger, Berman and Sturges (1916, pp. 15–33).

11. Omeliansky's Basal Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Potassium phosphate	0.5 g.
3. $MgSO_4$	0.03 g.
4. NaCl	0.5 g.
5. Na_2CO_3	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add one of the added nutrients as indicated under added nutrients.

Sterilization: The various media under 'added nutrients' are sterilized differently. The method for each medium will be given with the nutrient.

Use: To study nitrate and nitrite production from organic nitrogen. Author reported that in the concentrations used all tests for NH_3, nitrites or nitrates were negative. Nessler's reagent was used to determine ammonia amyl iodine reaction for nitrite and diphenylamine for nitrate.

Added nutrients: The author added one of the following materials to the basic solution:

(a) Asparagin 1.0%. Filter through a Chamberland filter to sterilize.

(b) Urea 1.0%. Filter through a Chamberland filter to sterilize.

(c) Bouillon 50.0 cc. Both basic solution and bouillon to be sterile. Method of sterilization not given.

(d) Egg white 1.0%. Sterilize in the steamer.

(e) Fresh urine 50.0 cc. Filter through a Chamberland filter to sterilize. The urine gave a slight ammonia test with Nessler's reagent. To remove all ammonia add 1.0% soda to the fresh urine, filter and store the filtrate for 2 days at 25°C. over H_2SO_4. Then filter through a Chamberland filter as above to sterilize.

Reference: Omeliansky (1899 p. 481).

12. Percival's Basal Solution

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	5.0 g.
3. $MgSO_4$	2.5 g.
4. NaCl	2.5 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add 10.0 g. of one of the added nutrients.

Sterilization: Not specified.

Use: To demonstrate ammonia production from protein.

Added nutrients:

(a) Percival added 1.0% of fibrin or gelatin.

(b) Harvey added 10.0 g. of one of the following materials: peptone, fibrin, gelatin.

(c) Omeliansky used 1.0 g. potassium phosphate, 0.5 g. $MgSO_4$, 0.1 g. NaCl in the basic solution and added one of the listed proteins. He studied the production of aroma by *Bact. esteroaromaticum.* Growth with aroma production took place with all materials except casein and keratin. One of the following materials was added:

egg albumin (Kahlbaum)	10.0 g.
blood albumin (Kahlbaum)	10.0 g.
peptone (Okuney)	10.0 g.
casein (Hammorsten) (Merck)	10.0 g.
keratin (Zyzkia, Merck)	10.0 g.

References: Percival (1920, p. 112) Harvey (1921–22, p. 102), Omeliansky (1923, pp. 409, 411).

13. Nawiasky's Basal Salt Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl	5.0 g.
3. K_2HPO_4	2.0 g.
4. $MgSO_4$	0.5 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Neutralize and make slightly alkaline

(3) Add one of the organic materials listed in added nutrients to (2).

Sterilization: Method not given.

Use: Cultivation of *Proteus vulgaris.*

Added nutrients:

asparagin	5.0 g.
glycocoll	4.0 g.
alanin	6.25 g.
isobutylaldehyde	1.6 g.
leucine	2.0 g.
aspartic acid	4.5 g.

Reference: Nawiasky (1908, p. 215).

14. Stoklasa's Basal Solution

Constituents:

1. Water (River, Tap)	1000.0 cc.
2. Dipotassium phosphate	0.5 g.
3. $CaCO_3$	1.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Add suitable carbon source.
(3) Distribute in large Erlenmeyer flasks (40 cm. x 15 cm.)

Sterilization: Sterilize in streaming steam or in the autoclave.

Uses: To study nitrogen assimilation by *Azotobacter* and *Radiobacter* as influenced by various carbohydrates.

Added nutrients: The following carbohydrates were suggested by the author to serve as carbon sources:

l-arabinose
l-xylose
d-glucose
d-galactose
d-fructose
saccharose
rhamnose
lactose
maltose

Reference: Stoklasa (1908 p. 491).

15. Heinze's Basal Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $CaCl_2$	0.2 g.
3. $MgSO_4$	0.4 g.
4. KH_2PO_4	2.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Add 1.0% of one of the listed added nutrients
(3) Adjustment of reaction not specified.

Sterilization: Not specified.

Use: To study oxalic acid formation. Oxalic acid was formed in quite small amounts. Heavy formation of HNO_3 and ammonia occurred.

Added nutrients: The author employed 1.0% of one of the following:

peptone
hemialbumose
gelatin

Reference: Heinze (1905, p. 18).

16. Söhngen's Basal Solution

Constituents:

1. Water	75.0 cc.
2. K_2HPO_4	0.05 g.
3. $CaCO_3$	

Preparation:

(1) Dissolve 2 in 1 and add 3.
(2) Place in a liter Erlenmeyer flask.
(3) Add suitable carbon source.

Sterilization: Method not specified.

Uses: To study the effect of filter paper (colloid) on bacterial processes, particularly fixation of atmospheric nitrogen.

Added Nutrients: The author employed the following:

(a) 1.0 g. glucose + 0.1 g. $CaCO_3$
(b) 1.0 g. glucose + 0.1 g. $CaCO_3$ + 10.0 g. filter paper cut in squares of 1 sq. cm.
(c) 1.0 g. mannitol + 0.1 g. $CaCO_3$
(d) 1.0 g. mannitol + 0.1 g. $CaCO_3$ + 10.0 g. filter paper

Reference: Söhngen (1913, p. 628).

17. van Delden's Basal Gypsum Solution

Constituents:

1. Water	1000.0 cc.
2. Gypsum	0.4 g.
3. K_2HPO_4	0.5 g.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Additional nutrients are added as indicated below.

Sterilization: Not specified.

Use: To study sulphate reduction by *Microspira desulfuricans.*

Added nutrients and variants: The author used one of the following combinations:

(a) Added 0.5 g. glucose and 0.5 g. peptone.
(b) Added 1.0 g. glucose, 1.0 g. $CaCO_3$, specified the use of tap water and used 1.2 g. gypsum.
(c) Specified the use of tap water, added 0.5 g. NH_4Cl, added 0.5 g. glucose and used 1.2 g. gypsum.
(d) Specified the use of tap water, added 0.5 g. sodium acetate, 0.5 g. asparagin and used 1.2 g. gypsum.
(e) Specified the use of tap water, added 1.0 g. asparagin, used 1.2 g. gypsum and added 1.25 g. of sodium succinate or potassium succinate or calcium citrate or potassium tartrate or potassium malate.

(f) Specified the use of tap water, added 1.0 g. asparagin and used 1.2 g. gypsum.

Reference: van Delden (1903–04, p. 83).

18. Czapek's Basal Solution (Waksman)

Constituents:

1. Distilled water	1000.0	cc.
2. K_2HPO_4	1.0	g.
3. KCl	0.5	g.
4. $MgSO_4$	0.5	g.
5. $FeSO_4$	0.01	g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: As desired.

Uses: Used by many investigators as a basic mineral or salt solution for comparison of availability of various nitrogen and carbon nutrients.

Reference: Waksman (1918, p. 479).

19. Buchanan's Basal Solution

Constituents:

1. Water	1000.0	cc.
2. KH_2PO_4	2.0	g.
3. $MgSO_4$ (0.01%)	0.1	g.

Preparation:

(1) Dissolve 2, 3 and one of the added nutrients in 1.

(2) Cool on ice.

(3) Filter to remove insoluble precipitates.

(4) Tube.

Sterilization: Intermittently in flowing steam on each of 3 successive days.

Use: To study growth of *Bacillus radicicola* bacteroids, and to study gum production by *Bacillus radicicola.* Author reported that generally carbohydrates and glucosides favored, while peptones inhibited the growth of *B. radicicola;* mannitol especially favored growth.

Added nutrients: One of the following materials or combinations was added:

Arabinose 2.0%
Rhamnose (Isodulcitol) 2.0%
Glucose 2.0%
  2.0% + peptone 1.0%
Mannose 2.0%
Galactose 2.0%
Levulose 2.0%
Sucrose 0.1, 10.0, 20.0, 30.0, or 50.0%
Sucrose 2.0% + peptone 1.0%
Sucrose 2.0% + KNO_3 0.05 to 0.5%
Sucrose 2.0% + nutrose 1.0%
Maltose 2.0% + sodium succinate 1.0%
Maltose 2.0% + sodium asparaginate 1.0%
Maltose 2.0% + ammonium citrate 1.0%
Maltose 2.0% + peptone 1.0%
Maltose 2.0% + asparagin 1.0%
Raffinose 2.0%
Melitose 2.0%
Inulin 2.0%
Glycerol 1.0, 3.0 or 5.0%
Glycerol 1.0, 3.0 or 5.0% + ammonium phosphate 0.5%
Inositol 2.0%
Mannitol 2.0%
Ammonium formate 1.0%, 2.0% or 5.0%
Ammonium Tartrate 1.0%
Ammonium citrate 1.0%
Sodium, potassium or calcium butyrate 0.5%, 1.0% or 2.0%
Sodium butyrate (0.5%) 5.0 g. + ammonium phosphate 0.5%
Calcium butyrate (1.0%) 1.0 g. + ammonium phosphate 0.5%
Potassium valerianate 0.5%, 1.0% or 2.0%
Sodium succinate 0.5%, 1.0% or 2.0%
Sodium succinate 1.0% + ammonium phosphate 0.5%
Calcium lactate 0.5%, 1.0% or 2.0%
Calcium lactate 1.0% + ammonium phosphate 0.5%
Sodium citrate 1.0%
Sodium citrate 1.0% + ammonium phosphate 0.5%
Asparagin 1.0%
Asparagin 1.0, 2.0, 3.0 or 5.0% + ammonium phosphate 0.5%
Asparagin 1.0% + sodium asparaginate 1.0%
Sodium asparaginate 1.0, 3.0 or 5.0% + ammonium phosphate 0.5%
Sodium asparaginate 0.5, 1.0, 3.0 or 5.0%
Nutrose 0.1, 0.5, 1.0, 2.0 or 5.0%
Nutrose 1.0% + ammonium tartrate 0.5%
Nutrose 1.0% + ammonium phosphate 0.5%
Nutrose 1.0% + asparagin 1.0%
Peptone (Witte) 1.0%
Peptone (Witte) 1.0% + Ammonium phosphate 0.5%
Peptone (Witte) 1.0% + sodium asparaginate 1.0%
Peptone (Witte) 1.0% + asparagin 1.0%

Peptone (Witte) 1.0% + KNO_3 0.1 or 1.0%
Amygdalin 2.0%
Amygdalin 2.0% + ammonium phosphate 0.5%
Salicin 2.0% + ammonium phosphate 0.5%
Salicin 2.0%
Ammonium phosphate

References: Buchanan (1909, pp. 382, 391, 62), Tanner (1909, p. 57)

20. Fuhrmann's Basal Solution

Constituents:

1. Water	1000.0	cc.
2. KH_2PO_4	1.0	g.
3. $CaCl_2$	0.1	g.
4. $MgSO_4 \cdot 7H_2O$	0.3	g.
5. NaCl	0.1	g.
6. Fe_2Cl_6	0.01	g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Distribute in 100.0 cc. lots.

Sterilization: Method not specified.

Uses: To study the availability of various nitrogen and carbon sources for *Pseudomonas cerevisiae.*

Added Nutrients: Make the following additions to each 100.0 cc.:

(a) Asparagin 1.0 g.
(b) Asparagin 1.0 g. + glucose 1.5 g.
(c) Asparagin 1.0 g. + sucrose 0.75 g.
(d) Potassium nitrate 1.0 g. + glycerol 1.0 g.
(e) Potassium nitrate 1.0 g. + sucrose 0.75 g.
(f) Potassium nitrate 1.0 g. + glucose 1.5 g.
(g) Ammonium chloride 1.0 g. + sucrose 0.75 g.
(h) Ammonium chloride 1.0 g. + glucose 1.5 g.

Reference: Fuhrmann (1906, p. 319).

21. Münter's Basal Salt Solution

Constituents:

1. Water	1000.0	cc.
2. $MgSO_4$	0.5	g.
3. NaCl	0.5	g.
4. $CaCl_2$	0.1	g.
5. K_2HPO_4	1.0	g.
6. $FeCl_3$	trace	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Neutralize with $CaCO_3$ and filter.
(3) Distribute in 100.0 cc. lots in Erlenmeyer flasks.
(4) Add 0.5 g. of one of the added nutrients to each flask.

Sterilization: Not specified.

Use: To study the availability of nitrogen for *Actinomycetes odorifer, Act. chromogenes, Act. albus I and II, Act. S. a, b, and c.* Good growth was obtained with albumin, hemi albumin, casein, asparagin, and alanine; little growth obtained with tyrosin, while generally no growth with urea, sulphocarbamide, or dicyandiamide.

Added nutrients: The author employed 0.5 g. of one of the following nitrogen sources for each 100.0 cc. of medium:

albumin
hemi albumin
casein
asparagin
urea
sulphocarbamide
alanine
tyrosine
dicyandiamide

Reference: Münter (1913 p. 373).

22. Meyer's Basal Salt Solution (Perotti)

Constituents:

1. Distilled water	1000.0	cc.
2. K_2HPO_4	1.0	g.
3. $CaCl_2$	0.1	g.
4. $MgSO_4$	0.1	g.
5. NaCl	0.1	g.
6. Iron chloride	0.01	g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Uses: Used by Perotti as a basis for the preparation of various solutions for the study of the dicyandiamid bacteria of the soil.

Variants:

(a) Buchanan used a basal solution containing the following materials:

1. Water	1000.0	cc.
2. KH_2PO_4	0.1	g.
3. $CaCl_2$	0.1	g.
4. $MgSO_4 \cdot 7H_2O$	0.3	g.
5. NaCl	0.1	g.
6. Fe_2Cl_6	0.01	g.

Buchanan added one of the following materials, and used the media to determine the constituents essential for the growth of *Bacillus thermophilus.* Growth was obtained with all materials except asparagin alone.

asparagin 1.0%
asparagin 1.0% + glycerol 1.0%

asparagin 1.0% + filter paper 1.0%
urea 1.0% + peptone 1.0%
peptone 1.0% + glucose
peptone 1.0% + lactose 1.0%
peptone 1.0% + sucrose 1.0%

(b) Klaeser added 2.0g KNO_3 and 10.0g. of glucose. He used the solution to study nitrate reduction.

(c) Stapp used 1.0g HK_2PO_4 instead of KH_2PO_4. He dissolved 3.0g Na_2HPO_4 and 0.5 g uric acid in 45.0 cc. water and added 50.0 cc. of the basal solution. The medium was used for the isolation of uric acid splitting bacteria from the feces and soil. The organisms studied were *Bact. cobayae*, *Bact. capri*, *Bact. guano*, *Bact. muscili*, *Bact. hollandicus*.

Reference: Buchanan (1906 p. 73). Perotti (1908 p. 220), Klaeser (1914 p. 38a) Stapp (1920 p. 3).

23. Dox's Inorganic Salt Solution For Fungi (Tanner)

Constituents:

1. Distilled Water	3000.0 cc.
2. $MgSO_4$	1.5 g.
3. K_2HPO_4	3.0 g.
4. KCl	1.5 g.
5. $FeSO_4$	0.03 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Uses: Cultivation of fungi.

Reference: Tanner (1919 p. 65).

24. Gage's Basal Salt Solution

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4	0.5 g.
3. $MgSO_4$	0.2 g.
4. $CaCl_2$	0.02 g.
5. $Fe_2(SO_4)_3$ 1 drop of 10% solution.	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Add 1.5 g. of suitable carbon source.

Sterilization: Method not specified.

Uses: Study of nitrogen fixation and of the morphology of the nitrogen fixing bacteria.

Added nutrients: The author suggests the addition of 1.5 g. of one of the following carbon sources:

Mannitol
Glucose
Dulcitol
Sorbitol
Sucrose
Galactose
Maltose
Mannan from Salep
Galacton from slippery elm.
Pentosan from dulse
Sinistrin
Lichenin

Reference: Gage (1910 p. 21).

25. Beijerinck and van Delden's Basal Solution

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4.7H_2O$	8.0 mg.
3. $MnSO_4.4H_2O$	0.5 mg.
4. $FeCl_3.3H_2O$	0.5 mg.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Uses: Used by Beijerinck and van Delden as a mineral mixture to be added in small quantities to a considerable variety of liquid media.

Reference: Beijerinck and van Delden (1903 p. 41).

SUBGROUP I-B. SECTION 2

Inorganic liquid media of known composition, nitrogen as free nitrogen only, complete solutions, used as media without addition of other nutrients.

"Complete" inorganic non-nitrogenous media have been described for the cultivation of certain iron bacteria and for certain algae. In each case it will be noted that either "well water" or a heavy inoculum of soil or soil extract is used. It is probable therefore that ammoniacal or nitrate nitrogen is usually present, and may in some cases be essential for the growth of the microörganisms.

The important media of this section may be differentiated as follows:

A_1. Medium employed primarily for iron bacteria.

Ellis' Ferric Hydroxide Solution	26

A_2. Media for algae. (Cyanophyceae or blue-green).

Beijerinck's Basal Phosphate Solution.	27
Heinze's Basal Solution A	28
Heinze's Basal Solution B	29

26. Ellis' Ferric Hydroxide Solution

Constituents:

1. Well water.
2. Ferric hydroxide.

Preparation: (1) Add freshly precipitated ferric hydroxide (amount not specified) to sterile well water in sterile flask.

Uses: Inoculate with *Spirophyllum ferrugineum* (one of the iron bacteria) and expose to sunlight.

Reference: Ellis (1907 p. 511).

27. Beijerinck's Basal Phosphate Solution

Constituents:

1. Water	100.0 cc.
2. K_2HPO_4	0.02 g.

Preparation:

(1) Dissolve 2 in 1.

(2) Place in a 3 liter flask and plug with cotton.

Sterilization: Not specified. For purposes used, probably generally unnecessary.

Uses: Add an inoculum of 1.0 or 2.0 g. garden earth and incubate at 16°–20°C. (In the light?) Oligonitrophilic organisms of the *Cyanophyceae* (blue-green algae) will develop, the medium becoming bluish green.

Variants: van Delden specified the use of "Grabenwasser."

Reference: Beijerinck (1901 p. 562), van Delden (1903–04 p. 85). Löhnis (1913 p. 115).

28. Heinze's Basal Solution A

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4	2.0 g.
3. $CaCl_2$	0.2 g.
4. $FeCl_3$ in 10% solution	20 drops.

Preparation:

(1) Dissolve 2, 3 and 4 in 1. If K_3PO_4 is used, the solution may be acidified with sulphuric acid and neutralized with soda.

(2) Distribute in 100 cc. lots.

Sterilization: Not specified.

Uses: Inoculate with 1.0 cc. of a suspension of 1.0 g. of soil in 100.0 g. sterile distilled water. Incubate (in the light?). The soil *Cyanophyceae* (blue green algae) will develop. Nitrogen may possibly be assimilated (fixed) in small amounts.

Variants: The author substituted K_2HPO_4 or K_3PO_4 for KH_2PO_4.

Reference: Heinze (1906 p. 703).

29. Heinze's Basal Solution B

Constituents:

1. Water	1000.0 cc.
2. Potassium phosphate neutral	0.2 g.
3. $MgSO_4$	0.2 g.
4. K_2SO_4	0.2 g.
5. $CaCO_3$	0.1 g.
6. Iron chloride	trace

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Uses: Inoculate with a suspension of soil. Soil algae, such as *Nostoc punctiforme* will develop. If soil bacteria are present *Nostoc punctiforme* will grow and nitrogen will be assimilated.

Reference: Heinze (1906 p. 653).

SUBGROUP I-B. SECTION 3

Inorganic liquid solutions of known composition; nitrogen supplied as ammonium salts; incomplete or basal solutions requiring the addition of other nutrients.

The several solutions falling in this section may be differentiated as follows:

A_1.* Containing ammonia as ammonium chloride.
  Boas' Basal Ammonium Chloride Solution 30
  Wherry's Basal Ammonium Chloride Solution B. 31
  Kendall, Walker and Day's Basal Ammonium Chloride Solution 32
  Löhnis' Basal Ammonium Chloride Salt Solution 33
A_2. Containing ammonia as ammonium sulphate.
B_1. Not containing additional salts.
  Buchanan's Basal Ammonium Sulphate Solution 34
  Cathelineau's Basal Ammonium Sulphate Solution 35
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30. Boas' Basal Ammonium Chloride Solution

Constituents:

1. Water	1000.0 cc.
2. NH_4Cl (0.25%)	2.5 g.
3. KH_2PO_4 (0.25%)	2.5 g.
4. $MgSO_4$ (0.15%)	1.5 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add 5.0% of one of the carbohydrates.

(3) Distribute in 40.0 cc. lots in 100.0 cc. Erlenmeyer jena glass flasks.

Sterilization: Not specified.

Uses: Used to study the formation of starch by molds, *Aspergillus oryzae*. If starch is formed, a blue color appears when iodine is added to the culture.

Added nutrients: The author used 5.0% of one of the following carbon sources:

sucrose
levulose
maltose
glucose
galactose

Reference: Boas (1922 p. 8).

31. Wherry's Basal Ammonium Chloride Solution B

Constituents:

1. Redistilled water	1000.0 cc.
2. Na_2CO_3	1.0 g.
3. KCl	1.0 g.
4. $CaCl_2$	1.0 g.
5. $MgSO_4$	1.0 g.
6. NH_4Cl	2.0 g.
7. KH_2PO_4	2.0 g.

Preparation:

(1) Dissolve 2, 3, and 4 in 1. This is called solution "B".

(2) Dissolve 6 and 7 in (1).

(3) Distribute in 50 cc. lots and add one of the test materials to each lot.

Sterilization: Not specified.

Use: To study acid fastness of tubercle bacilli. Growth started first on methyl alcohol. After 20 days the order of vigor of growth was as follows: Propyl, butyl, ethyl and methyl alcohol. Culture became strongly acid fast only in propyl alcohol.

Added nutrients: The following carbon sources were used:

ethyl alcohol	2.0%
propyl alcohol	2.0%
amyl alcohol	2.0%
butyl alcohol	2.0%
methyl alcohol	2.0%
leucine	0.1 g.
tyrosine	0.1 g.
alanine	0.1 g.
glycocoll	0.1 g.
aspartic acid	0.1 g.
glutamic acid	0.1 g.

(Salts to be Kahlbaum C. P.)

Reference: Wherry (1913 p. 116).

32. Kendall, Walker and Day's Basal Ammonium Chloride Solution

Constituents:
1. Redistilled water............1000.0 cc.
2. NH_4Cl...................... 4.0 g.
3. $NaCl$........................ 5.0 g.
4. Na_2HPO_4

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Add one of the nutrients listed below.
(3) Distribute in 100.0 cc. quantities in flasks.

Sterilization: Method not given.

Use: To study lipase production by tubercle bacilli. Different esters were added to clear bacteria free culture broth and incubated for 24 hours. Amount of acid produced measured in terms of N/50 NaOH, determined lipase production.

Added nutrients: The authors used ethyl alcohol, glycerol or mannitol as carbon sources.

Reference: Kendall, Walker and Day (1914 p. 455).

33. Löhnis' Basal Ammonium Chloride Solution

Constituents:
1. Water (tap)................1000.0 cc.
2. NH_4Cl...................... 0.5 g.
3. K_2HPO_4.................... 0.5 g.

Preparation:
(1) Dissolve 2 and 3 in 1.
(2) Add 1.0% of one of the listed nutrients

Sterilization: Not specified.

Use: Methane production by cellulose decomposers.

Added nutrients: The author added 1.0% of one of the following materials:

acetate
butyrate
lactate
gum
starch
sugar
peptone
wool

Reference: Löhnis (1913 p. 93).

34. Buchanan's Basal Ammonium Sulphate Solution

Constituents:
1. Water......................1000.0 cc.
2. $(NH_4)_2SO_4$ (0.5%).......... 5.0 g.

Preparation:
(1) Add 2.0% of one of the carbon sources to water and dissolve.
(2) Then add 0.5% ammonium sulphate to (1).

Sterilization: Sterilize on each of 3 successive days for 20 minutes in flowing steam.

Use: To study gum production of *Bacillus radicicola.*

Added nutrients: The following carbon sources (2.0%) were used by the author:

arabinose
rhamnose
glucose
mannose
galactose
levulose
melitose
inositol
sucrose
maltose
lactose
raffinose
mannitol
inulin
starch

Reference: Buchanan (1909 p. 388).

35. Cathelineau's Basal Ammonium Sulphate Solution

Constituents:
1. Water.......................100.0 cc.
2. $(NH_4)_2SO_4$.................. 1.0 g.

Preparation:
(1) Dissolve 2 in 1.
(2) Add one of the following to (1).
(a) Glucose.................... 5.0 g.
(b) Sodium succinate.......... 5.0 g.
(c) Sodium phosphate.......... 5.0 g.
(d) Sodium succinate.......... 5.0 g.
Glucose.................. 5.0 g.
(e) Glucose.................... 5.0 g.
Sodium phosphate........ 5.0 g.
(f) Sodium succinate.......... 5.0 g.
Sodium phosphate........ 5.0 g.

Sterilization: Method not given.

Use: To study pigment production and fluorescence by *Bacillus viridis* (Lesage). Pigment production was independent of the amount of phosphates present.

Added nutrients: The author added the following carbon sources and salts:
(a) Glucose...................... 5.0 g.
(b) Sodium succinate............ 5.0 g.
(c) Glucose...................... 5.0 g.
Sodium succinate........... 5.0 g.
(d) Glucose...................... 5.0 g.
Sodium phosphate.......... 5.0 g.
(e) Sodium succinate............ 5.0 g.
Sodium phosphate.......... 5.0 g.

Reference: Cathelineau (1896 p. 235).

36. Péré's Basal Ammonium Sulphate Solution

Constituents:
1. Water.......................100.0 cc.
2. Ammonium phosphate........ 1.0 g.

3. $(NH_4)_2SO_4$ .... 0.5 g.
4. Potassium phosphate .... 0.25 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Add one of the test materials in amounts given to (1).
(3) The reaction must be neutral. If acid after sterilization, add a few drops of ammonia.

Sterilization: Method not given.

Use: To study the decomposition of carbonaceous bodies (polyatomic alcohols, starch, etc.) by *Tyrothrix tenuis*, *Bacillus mesentericus vulgatus* and *Bacillus subtilis*.

Added nutrients: The author used the following substances:

Mannitol .... 10.0 g.
Glycerol .... 5.0 g.
Glycerose, amount not given
Potato starch .... 2.0 g.
Sucrose .... 5.0 g.
Glucose .... 5.0% = 5.0 g

Reference: Péré (1896 p. 421).

37. Henneberg's Basal Ammonium Sulphate Solution

Constituents:

1. Water .... 1000.0 cc.
2. KH_2PO_4 .... 3.0 g.
3. $(NH_4)_2SO_4$ .... 3.0 g.
4. $MgSO_4$ .... 2.0 g.
5. Carbon source.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Do not adjust the reaction.
(3) Add any desired carbon source.

Sterilization: Not specified.

Use: To study a suitable carbon source for acetic acid organisms, *B. pasteurianum*, *B. aceti*, *B. oxydans* and *B. Kützingianum*. Glucose was found to be a suitable carbon source, but methyl, ethyl or propyl alcohol, acetic acid or ammonium tartrate were not suitable sources of carbon.

Reference: Henneberg (1898 p. 18).

38. Omeliansky's Basal Ammonium Sulphate Solution

Constituents:

1. Distilled water .... 1000.0 cc.
2. Potassium phosphate .... 0.1 g.
3. $(NH_4)_2SO_4$ .... 0.1 g.
4. $MgSO_4$ .... 0.05 g.
5. $NaCl$ .... Trace
6. Chalk

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Prepare a 1.0% solution of one of the test materials.
(3) Distribute in test tubes.
(4) Add chalk to each tube.

Sterilization: Not specified.

Use: To study the fermentation ability of *Bacterium formicicum* under either anaerobic or aerobic conditions.

Added nutrients: The author used 10 grams of the following carbon sources:

Glucose
Sucrose
Galactose
Lactose
Mannitol
Dulcitol
Maltose
Erythritol
Dextrin
Amylum
Inulin
Arabinose
Gum arabic
Ethylene glycol
Glycerol

Reference: Omeliansky (1903–4 p. 258).

39. Kita's Basal Ammonium Sulphate Solution

Constituents:

1. Water .... 1000.0 cc.
2. $MgSO_4$ .... 0.25 g.
3. KH_2PO_4 .... 5.0 g.
4. $FeCl_3$ solution drops
5. $(NH_4)_2SO_4$ .... 5.0 g.

Preparation:

(1) Dissolve 2, 3 and 5 in 1.
(2) Add several drops of a ferric chloride solution to (1).
(3) Add 50.0 g. of any desired carbohydrate to (2).

Sterilization: Not specified.

Use: To study utilization of various carbon sources by molds, *Aspergillus okazaki*, *Aspergillus candidus*, *Aspergillus albus*, *Aspergillis tamarii*, *Pseudorhizopus*, *Aspergillus glaucus*.

Reference: Kita (1913 p. 434).

40. Grimm's Basal Ammonium Sulphate Solution

Constituents:

1. Distilled water .... 1000.0 cc.
2. K_2HPO_4 .... 0.5 g.
3. $MgSO_4$ .... 0.5 g.
4. $(NH_4)_2SO_4$ .... 5.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Distribute in 30.0 cc. lots in 200.0 cc. Erlenmeyer flasks.

(3) After sterilization add one of the test materials to (2). (Amount not specified).

Sterilization: Not specified.

Use: To study the utilization of various carbon sources by *Oospora lactis* and *Aspergillus repens.* Growth with ethyl alcohol, isobutyl alcohol, acetic acid, ethyl ether, propyl, isopropyl and amyl formic acid, methyl, ethyl, propyl, isopropyl, isobutyl and amyl acetic acid. Other materials give very little or no growth.

Added nutrients: The author used the following carbon sources:

Pentan
Heptan
Hexan
Octan
Methyl alcohol
Ethyl alcohol
Propyl alcohol
Isopropyl alcohol
Butyl alcohol
Isobutyl alcohol
Amyl alcohol
Formic acid
Acetic acid
Propionic acid
Butyric acid normal
Isobutyric acid
Valeric acid
Ethyl ether
Propyl ether
Formaldehyde
Acetaldehyde
Propionic aldehyde
Methyl formic acid
Ethyl formic acid
Propyl formic acid
Isobutyl formic acid
Methyl acetic acid
Ethyl acetic acid
Propyl acetic acid
Isobutyl acetic acid
Methyl iodine
Ethyl iodine
Ethyl bromide
Propyl bromide
Chloroform

Reference: Grimm (1914 p. 648).

41. Waksman and Joffe's Basal Sulphur Ammonium Sulphate Solution

Constituents:

1. Distilled water	1000.0	cc.
2. $(NH_4)_2SO_4$	2.0	g.
3. K_2HPO_4	1.0	g.
4. $MgSO_4$	0.5	g.
5. KCl	0.5	g.
6. $FeSO_4$	0.01	g.
7. Sulfur	10.0	g.
8. $Ca_3(PO_4)_2$,	2.5 or 10.0	g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8 and one of the added nutrients in 1.

(2) Distribute in 100.0 cc. portions in 250.0 cc. flasks.

Sterilization: Sterilize in flowing steam for 30 minutes on 3 consecutive days.

Use: Enrichment and isolation of sulphur bacteria, *Thiobacillus thiooxidans.* If glucose be omitted fungi do not grow. Medium supports the growth of all sulphur oxidizers.

Added nutrients:

(a) The authors added 10.0 glucose or any other organic or inorganic stimulator.

(b) Used the basic solution without any additions.

Reference: Waksman and Joffe (1922 p. 239).

42. Bokorny's (Vierling) Basal Ammonium Sulphate Solution

Constituents:

1. Water	1000.0	cc.
2. $(NH_4)_2SO_4$	1.0	g.
3. KH_2PO_4	0.2	g.
4. $MgSO_4$	0.1	g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add 2.0 g. of one of the carbon sources

Sterilization: Not specified.

Use: Author used the solution to study urease production.

Added nutrients: The author added the following carbon sources:

Glycerol
Ethyl alcohol
Lactose
Galactose
Rhamnose
Arabinose
Sorbose
Xylose
Mannose

Reference: Vierling (1920 p. 31).

43. Verkade and Söhngen's Basal Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4 (0.05%)	0.5 g.
3. $MgSO_4$ (0.05%)	0.5 g.
4. $FeCl_3$ (0.01%)	0.1 g.
5. $MnSO_4$ (0.01%)	0.1 g.
6. $(NH_4)_2SO_4$ (0.05%)	0.5 g.
7. $CaCO_3$	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Add 0.25% of one of the test materials and an excess of $CaCO_3$.

Sterilization: Autoclave at 110°C. for 10 minutes.

Use: To study the availability of organic acids as a carbon source for *Aspergillus niger* and *Penicillium glaucum.* Fumaric, citric aconitic, oleic and erucic acids give good growth.

Added nutrients:

maleic acid
fumaric acid
citric acid
itaconic acid
phenylitaconic acid
allocinnamic acid
cinnamic acid
aconite acid
isocrotonic acid
crotonic acid
BB-dimethylacrylic acid
angelic acid
tiglic acid
oleic acid
elaidic acid
erucic acid
brassidic acid

Variants: The authors substituted 0.05% KNO_3 for $(NH_4)_2SO_4$.

Reference: Verkade and Söhngen (1920 p. 82).

44. Starkey's Basal Ammonium Sulphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $(NH_4)_2SO_4$	0.2 to 0.4 g.
3. KH_2PO_4	3.0 to 4.0 g.
4. $CaCl_2$	0.25 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.01 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Add one of the added nutrients.

Sterilization: Heat in flowing steam for 30 minutes on each of three consecutive days.

Added nutrients: The author added one of the following:

(a) Sulphur 1.0%. The sulphur was weighed out and placed in individual flasks, the basic solution added and sterilized.

(b) Sodium thiosulphate 0.5%. The thiosulphate was dissolved in water, sterilized and added to the sterile basic solution in the necessary amounts.

Use: Cultivation of *Thiobacillus thioxidans* and to study oxidation of sulphur.

Reference: Starkey (1925 p. 138).

45. Ayers and Rupp's Basal Sodium Ammonium Phosphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Sodium ammonium phosphate	1.5 g.

Preparation:

(1) Dissolve 2 in 1.

(2) Add one of the combinations given under added nutrients.

(3) Adjust to pH = 7.0

Sterilization: Not specified.

Use: To study acid and alkali production.

Added nutrients and modifications: The author added one of the following combinations:

(a)	Glucose	5.0 g.
	citric acid (neutralized with NaOH)	2.5 g.
	KCl	0.2 g.
(b)	glucose	5.0 g.
	KCl	0.2 g.
(c)	citric acid (neutralized with NaOH)	2.5 g.
	KCl	0.2 g.

(d) Used 3.6 g. sodium ammonium phosphate in the basic solution and added KH_2PO_4 1.2 g. and glucose 5.0 g. To this solution was added 1.2 g. of one of the following acids neutralized with NaOH: formic acid, acetic acid, lactic acid, succinic acid.

(e) Same as (d) without the addition of acids.

Reference: Ayers and Rupp (1918 pp. 199, 209).

46. Committee S. A. B. Basal Ammonium Phosphate Solution

Constituents:
1. Water........................1000.0 cc.
2. $(NH_4)H_2PO_4$................ 1.0 g.
3. KCl......................... 0.2 g.
4. Brom Cresol Purple (Saturated aqueous solution)..... 2.0 cc.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Adjust to pH = 7 by addition of NaOH.
(3) Add fermentable substances.
(4) Tube.

Sterilization: Method not specified.

Uses: To test the ability of microörganisms to produce acid in a solution having ammonia as the nitrogen source and a suitable fermentable carbon compound.

Added nutrients: Suitable amounts of any of the carbohydrates or of the polyatomic alcohols, etc., may be used.

Variant. Authors also prepared the medium as follows:
(1) Dissolve 1.0 g. $(NH_4)H_2PO_4$, 0.2 g. NaCl, 0.2 g. $MgSO_4$ and 10.0 g. of one of the added nutrients in 1000.0 cc. water.
(2) Adjust to pH = 7.0 by the addition of NaOH.
(3) Indicator may be added:
Litmus
Phenol red, 2.0 cc. saturated aqueous solution per liter
Brom cresol purple, 2.0 cc. saturated aqueous solution per liter
Brom cresol purple, 1.0 cc. saturated aqueous solution per liter
Cresol red, 1.0 cc. saturated aqueous solution per liter
Brom cresol green 2.0 cc. of a 2.0% alcoholic solution per liter
Brom-chlor phenol blue 5.0 cc. of saturated aqueous solution per liter

Reference: Committee Society of American Bacteriologists (1922 p. 523), (1923 p. 11).

47. Kendall, Day and Walker's Basal Ammonium Phosphate Solution

Constituents:
1. Redistilled water............1000.0 cc.
2. $(NH_4)_2HPO_4$................ 4.0 g.
3. NaCl........................ 5.0 g.

Preparation:
(1) Dissolve 2 and 3 in 1.
(2) Add one of the added nutrients listed below.
(3) Distribute in 100.0 cc. lots.

Sterilization: Not specified.

Use: To study metabolism and lipase production by tubercle bacilli. Authors used alizarin, neutral red and phenolphthalein to study reaction changes. Ziehl-Nielsen stain for staining. Different esters were added to clear bacteria free culture broth, incubated for 24 hours and the amount of acid produced, (measured in terms of N/50 NaOH) determined lipase production.

Added nutrients: The authors added one of the following nutrients:
mannitol.........................10.0 g.
glycerol.........................30.0 g.
sodium acetate

Reference: Kendall, Day and Walker (1914 p. 434), Kendall, Walker and Day (1914 p. 455).

48. Laurent's Basal Ammonium Sulphate Solution

Constituents:
1. Water........................1000.0 cc.
2. Ammonium phosphate (neutral)........................ 2.5 g.
3. Potassium phosphate (neutral)........................ 2.5 g.
4. $MgSO_4$..................... 1.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: As desired; not specified.

Uses: Used with the addition of 1.0% of any one of a considerable variety of nitrogenous and carbon compounds.

Added nutrients:
(a) The following were employed:
saccharose
lactose
glucose
mannite
glycerine
peptone
asparagin
potassium succinate
potassium lactate
potassium citrate
potassium tartrate
ammonium bimalate
sodium butyrate
sodium hippurate
sodium formate
potassium acetate

These were used by Laurent in his

studies of *Bacillus fluorescens putidus.*

(b) Laurent cultivated Sclerotinia Libertiana using the basic solution and adding 1.0% peptone, 2.5% lactose, 2.5% glycerol and 2.5% potassium tartrate.

Variants: Smith cultivated plant parasites on a medium composed of 2.0 g K_2HPO_4, 0.1 g ammonium phosphate, 0.19 $MgSO_4$, and used 5.09 sodium formate or acetate as an added nutrient.

Reference: Laurent (1899 p. 43, 81). Smith (1905 p. 50).

49. Fermi's Basal Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. Ammonium Phosphate (0.5 to 1.0%)	5.0 to 10.0 g.
3. KH_2PO_4 (0.5%)	5.0 g.
4. $MgSO_4$ (0.5%)	5.0 g.
5. K_3PO_4 (0.05%)	0.5 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add one of the added nutrients.

Sterilization: Not specified.

Use: Cultivation of schizomycetes.

Added nutrients: The author added 5.0% glycerol or 5.0% sucrose.

Variants: Tanner gave the following as Fermi's Solution:

1. Distilled water	1000.0 cc.
2. $MgSO_4 \cdot 7H_2O$	0.2 g.
3. K_2HPO_4	1.0 g.
4. $(NH_4)_2HPO_4$	10.0 g.
5. Glycerol	45.0 g.

Reference: Fermi (1892 p. 26), Tanner (1919 p. 68).

50. Palladin's Basal Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. Ammonium phosphate	4.7 g.
3. Potassium phosphate	3.0 g.
4. $MgSO_4$	1.0 g.
5. $CaCl_2$	1.0 g.
6. $FeCl_2$	Trace

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Add sufficient carbohydrate to make solution one fourth molar.

(3) Distribute in thin layers in Erlenmeyer flasks.

Sterilization: Method not specified.

Use: Cultivation of *Chlorothecium saccharophilum.*

Variants: The author suggests the following carbon sources:

saccharose
mannitol
glucose
raffinose

Reference: Palladin (1903 p. 146).

51. Schukow's Basal Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. Ammonium phosphate	5.0 g.
3. Potassium phosphate	1.0 g.
4. $MgSO_4$	0.5 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add 9.0 to 10.0% of one of the organic acids to (1).

(3) Adjustment of reaction not specified.

(4) Distribute into fermentation flasks (sealed with H_2SO_4).

(5) After inoculation plug the flask with a cork and seal with paraffin.

Sterilization: Sterilize in a steamer (method not given).

Use: To study the utilization of acids by yeast. The author found 0.1 g. tartaric, 0.15 g. malic and 0.26 g. citric acid was used in 65 days by the yeast. Acid determinations were made by titration with normal NaOH, using litmus as an indicator.

Added nutrients and variants:

(a) The author added 9.0 to 10.0% of one of the following organic acids:
tartaric malic citric

(b) Henneberg specified the use of 0.3% KH_2PO_4 and used 0.1% $MgSO_4$ instead of 0.05%. He added 5.0% glucose and used it for the cultivation of lactic acid bacteria.

Reference: Schukow (1896 p. 607), Henneberg (1903 p. 7).

52. Koser's Basal Ammonium Phosphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. NaCl	5.0 g.
3. $MgSO_4 \cdot 7H_2O$	0.2 g.

4. $(NH_4)H_2PO_4$	1.0 g.
5. K_2HPO_4	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and one of the test materials in 1. Solution is clear at pH = 6.7 to 6.8.

(2) Add one of the test materials. Add the different organic acids to (1) in such amounts so that when brought to pH reading of 6.8 by addition of NaOH the resulting concentration of the Na salt will be approximately 0.2%.

(3) Tube in 8 to 10 cc. lots.

Sterilization: Autoclave at 15 pounds pressure for 15 minutes.

Use: To study the utilization of organic salts and acids by the colon-aerogenes group. Glucose and lactose were added as controls to determine if the solution would support the growth of the organism.

Added nutrients: The author added approximately 0.2% of the sodium salt of the following acids. In case of citric acid 0.1 to 0.5% was used.

glucose 0.2%
lactose 0.2%
sodium salt of one of the organic acids
acetic
propionic
n-valeric
n-butyric
iso-valeric
n-caproic
succinic
malic
lactic
glyceric
citric
tartaric
mucic
malonic
oxalic
benzoic
salicylic
o-phthalic

Reference: Koser (1923 p. 497).

53. Münter's Basal Ammonium Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4$	0.5 g.
3. NaCl	0.5 g.
4. $CaCl_2$	0.2 g.
5. K_2HPO_4	1.0 g.
6. NH_4NO_3	
7. $CaCO_3$	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Neutralize by the addition of $CaCO_3$.

(3) To each 100.0 cc. of (2) add 0.025 g. of nitrogen in the form of NH_4NO_3.

(4) Add 1.0 g. of one of the test materials to (3).

Sterilization: Not specified.

Uses: To study the availability of various carbon sources for *Actinomyces odorifer*, *Act. chromogenes*, *Act. albus* and others. Glycerin laevulose, dextrose, galactose, mannite and starch were good carbon sources for all the actinomycetes studied. The remaining carbohydrates serve as suitable carbon sources for some of the actinomyces but not all. Oxalic acid, tartaric acid, and hippuric acid are not suitable carbon sources for the actinomycetes studied. The remaining acids are generally fair carbon sources for most of the actinomycetes studied.

Added nutrients: The author used the following carbon sources:

sucrose
mannitol
galactose
lactose
inulin
glucose
arabinose
laevulose
glycerol
starch

Variants: The author omitted the $CaCl_2$ and $CaCO_3$ and used one of the following carbon sources. The acid was neutralized and made slightly alkaline by the addition of Na_2CO_3 or $CaCO_3$. 0.025 g. nitrogen in the form of NH_4NO_3 and 0.25 g. of the test material was added to each 50.0 cc. of medium.

oxalic acid
acetic acid
succinic acid
malic acid
tartaric acid
zitric acid
hippuric acid
uric acid
humus acid
aspartic acid

Reference: Münter (1913 pp. 368, 371).

54. von Bronsart's Basal Ammonium Nitrate Solution

Constituents:

1. Water	1000.00 cc.
2. $MgSO_4$ (0.25%)	2.5 g.
3. KH_2PO_4 (0.25%)	2.5 g.
4. NH_4NO_3 (1.0%)	10.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add one of the test materials to (1).

Sterilization: Not given.

Use: To study utilization of various carbon sources by *Xylaria*, *Xylaria arbuscula*, *Xylaria polymorpha*, *Xylaria hypoxylon*. Growth best using starch as a carbon source with *Xylaria arbuscula*, and levulose produces best growth with

Xylaria polymorpha, *Xylaria hypoxylon* growth very poor in any of the solutions.

Added nutrients: The author used the following carbon sources:

Starch	levulose
inulin	glucose
dextrin	mannitol

Reference: von Bronsart (1919 p. 57).

55. Adolf Mayers' Basal Ammonium Nitrate Solution (Smith)

Constituents:

1. Distilled water	1000.0 cc.
2. $MgSO_4$	10.0 g.
3. NH_4NO_3	15.0 g.
4. $Ca_3(PO_4)_2$	0.1 g.
5. Potassium phosphate	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and one of the added nutrients in 1.

Sterilization: Not specified.

Use: Study of phosphorescent and acid forming bacteria.

Variants: Add 3.0% NaCl if luminous bacteria are to be cultivated, and an excess of pure $CaCO_3$ if acid forming bacteria are to be grown.

Added nutrients: Smith added any desired carbohydrate, alcohol, etc.

Reference: Smith (1905 p. 197).

56. Prazmowski's Basal Ammonium Carbonate Solution (Smith)

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	5.0 g
3. $MgSO_4$	5.0 g.
4. $(NH_4)_2CO_3$	5.0 g.
5. $CaCl_2$	0.5 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and one of the added nutrients in 1.

Sterilization: Not specified.

Use: Study of phosphorescent and acid forming bacteria.

Added nutrients: Smith added any desired carbohydrate, alcohol, etc.

Reference: Smith (1905 p. 197).

SUBGROUP I-B. SECTION 4

Inorganic liquid media of known composition; nitrogen supplied as ammonium salts; complete nutrient solutions primarily for study of organisms oxidizing ammonia to nitrites.

A_1.* Ammonia present as ammonium chloride.
  Hewlett's Ammonium Chloride Solution (Johnson)........ 57
  Beijerinck and Minkman's Ammonium Chloride Solution........ 58
A_2. Ammonia present as ammonium carbonate.
  Prazmowski's Ammonium Carbonate Solution. (Tanner)........ 59
A_3. Ammonia present as ammonium sulphate.
B_1. Without additional salts.
  Wimmer's Ammonium Sulphate Solution........ 60
B_2. With other salts.
C_1. Magnesium carbonate added.
D_1. Phosphates not added.
  Winogradsky's Ammonium Sulphate Solution (Harvey)........ 61
D_2. Phosphates added.
E_1. All additional salts of mono valent cations.
  Buhlert and Fickenday's Ammonium Sulphate Solution........ 62
E_2. Additional salts of mono and di valent cations.
F_1. Calcium salts present.
  Winogradsky's Ammonium Sulphate Solution........ 63
  Giltner's Modified Winogradsky's Ammonium Sulphate Solution........ 64
F_2. Calcium salts not present.
  Winogradsky and Omeliansky's Ammonium Sulphate Solution........ 65
E_3. Containing Additional salts of trivalent cations.
  Gibbs' Ammonium Sulphate Solution. (modified by Gowda)........ 66
C_2. Magnesium carbonate not added.
D_1. Calcium carbonate, chalk or lime added.
E_1. Magnesium sulphate added.
  Omeliansky's Ammonium Sulphate Solution. (Harvey)........ 67
E_2. Magnesium sulphate not added.
  Christensen's Ammonium Sulphate Solution........ 68
D_2. Calcium carbonate chalk or lime not added.
  Gage's Ammonium Sulphate Solution........ 69

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* See A_2 A_3 and A_4.

Omeliansky's Ammonium Sulphate Solution. (Löhnis-Arnd)........... 70
Löhnis' Ammonium Sulphate Solution................................ 71

A_4. Ammonia present as a salt of phosphoric acid.

Stutzer's Ammonium Magnesium Phosphate Solution................. 72

57. Hewlett's (Johnson) Ammonium Chloride Solution.

Constituents:

1. Distilled water	1000.0 cc.
2. NH_4Cl	0.5 g.
3. Potassium phosphate	0.1 g.
4. $MgSO_4$	0.02 g.
5. $CaCl_2$	0.01 g.
6. $CaCO_3$	5.0 g.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Add 6 and distribute into flasks.

Sterilization: Not specified.

Use: For the study of nitrite formation.

Reference: Johnson (1912 p. 219).

58. Beijerinck and Minkman Ammonium Chloride Solution

Constituents:

1. Water	100.0 cc.
2. K_2HPO_4	0.02 g.
3. NH_4Cl	0.02 g.
4. $NaHCO_3$	0.1 g.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Distribute into Söhngen's culture apparatus.

Sterilization: Not specified.

Use: Inoculate with soil for study of formation of nitrites. Cultivation of *Bacillus sausseri*.

Reference: Beijerinck and Minkman (1910 p. 60).

59. Prazmowski's Ammonium Carbonate Solution (Tanner)

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	5.0 g.
3. $MgSO_4 \cdot 7H_2O$	5.0 g.
4. $(NH_4)_2CO_3$	5.0 g.
5. $CaCl_2$	0.5 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Reference: Tanner (1919 p. 63).

60. Wimmer's Ammonium Sulphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $(NH_4)_2SO_4$	2.0 or 4.0 g.

Preparation: (1) Dissolve 2 in 1.

Sterilization: Not specified.

Use: For study of nitrification (production of nitrites) by soil bacteria.

Reference: Wimmer (1904 p. 140).

61. Winogradsky Ammonium Sulphate Solution (Harvey)

Constituents:

1. Distilled water	1000.0 cc.
2. $(NH_4)_2SO_4$	1.0 g.
3. K_2SO_4	1.0 g.
4. $MgSO_4$	7.5 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Study of nitrifying organisms.

Reference: Harvey (1921 p. 106).

62. Buhlert and Fickendey Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2SO_4$	4.0 g.
3. Potassium phosphate	2.0 g.
4. Basic magnesium carbonate ($4MgCO_3 \cdot Mg(OH)_2 \cdot 5H_2O$)	40.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Study of nitrifying bacteria of the soil.

Variants:

(a) Fremlin's (described by Gage) solution is similar; the potassium phosphate is specified as KH_2PO_4, and the proportions as follows:

1. Water (nitrite free)	1100.0 g.
2. KH_2PO_4	1.0 g.
3. $(NH_4)_2SO_4$	1.0 g.
4. $MgCO_3$	1.0 g.

2 and 3 dissolved in 1000.0 cc. water and 4 in 100.0 cc. water. These solutions are sterilized separately and mixed aseptically.

(b) Percival distributed the solution in 25.0 cc. lots, and added 20.0 cc. of a mixture of equal parts soil and sterile tap water to each lot.

(c) Abbott gave the following solution:

1. Water	1000.0 cc.
2. $(NH_4)_2SO_4$	1.0 g.
3. Potassium phosphate	1.0 g.
4. $MgCO_3$	(Basic)

(1) Dissolve 2 and 3 in 1.
(2) Flask in 100.0 cc. quantities.
(3) Add 0.5 to 1.0 g. of basic $MgCO_3$ suspended in a little sterile water to each flask.

References: Buhlert and Fickendey (1906 p. 404), Barthel (1910 p. 112), Percival (1920 p. 208), Gage (1910 p. 14), Abbott (1921 p. 603).

63. Winogradsky's Ammonium Sulphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $MgSO_4$	0.5 g.
4. $CaCl_2$	trace
5. $MgCO_3$	amount not specified
6. $(NH_4)_2SO_4$	2.0 to 2.5 g.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Distribute in flasks.
(3) After sterilization add $MgCO_3$ (amount not specified) and from 2 to 2.5 parts per thousand of $(NH_4)_2SO_4$.

Sterilization: Method not specified.

Use: To show nitrification by organisms found in the soil. Inoculate with soil.

Variants:

(a) Burri and Stutzer

1. Water	1000.0 cc.
2. NaCl	0.5 g.
3. KH_2PO_4	1.0 g.
4. $MgSO_4$	0.5 g.
5. $CaCl_2$	trace
6. $(NH_4)_2SO_4$	2.0 g.
7. $MgCO_3$	

(b) Heinemann

1. Water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $MgSO_4$	0.5 g.
4. $CaCl_2$	0.01 g.
5. NaCl	2.0 g.
6. $MgCO_3$	
7. $(NH_4)_2SO_4$	2.0 g.

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Distribute in 20.0 cc. lots.
(3) Add several tenths grams of $MgCO_3$ to each lot.
(4) Sterilize intermittently.
(5) Add 1.0 or 2.0 cc. of a sterile 2.0% $(NH_4)_2SO_4$ solution to each lot under aseptic conditions.

(c) Harvey modified Burri and Stutzer's solution in that he used 1.0 g. K_2HPO_4 instead of KH_2PO_4 and used 2.0 g. NaCl instead of 0.5 g.

References: Winogradsky (1891 p. 577), Burri and Stutzer (1896 p. 106), Harvey (1921–22 p. 105) Heinemann (1905 p. 129).

64. Giltner's Modified Winogradsky's Ammonium Sulphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $(NH_4)_2SO_4$	0.4 g.
3. $MgSO_4$	0.05 g.
4. K_2HPO_4	0.1 g.
5. Na_2CO_3	0.6 g.
6. $CaCl_2$	trace

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Study of nitrate formation.

Reference: Giltner (1921 p. 369).

65. Winogradsky and Omeliansky's Ammonium Sulphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $(NH_4)_2SO_4$	2.0 g.
3. Potassium phosphate	1.0 g.
4. $MgSO_4$	0.5 g.
5. NaCl	2.0 g.
6. $FeSO_4$	0.4 g.
7. $MgCO_3$	in excess

Preparation:
(1) Dissolve 3, 4, 5 and 6 in 1.
(2) Distribute in 50.0 cc. lots.
(3) Add $MgCO_3$ in excess to each flask.
(4) After sterilization add 1.0 cc. of a sterile 1.0% solution of pure $(NH_4)_2SO_4$ under aseptic conditions.

Sterilization: Method not specified.

Use: To study nitrification by soil bacteria.

Variants: The appended table gives combinations used by various authors. The preparation of these variants is practically the same as for the original. Omeliansky found that repeated inoculation from this medium into the same medium usually yielded a pure culture of nitrifying organisms when initially inoculated with soil. Giltner and Löhnis used Harvey's solution (1.0 g. $(NH_4)_2SO_4$) and suggested the use of either $CaCO_3$ or $MgCO_3$. He reported that both nitrite and nitrate bacteria are stimulated if $CaCO_3$ is used instead of $MgCO_3$. Tanner used KH_2PO_4 in Harvey's (a) solution instead of K_2HPO_4.

(3) Distribute (1) in 5.0 cc. lots.
(4) Add 0.5 cc. of (2) to each 5.0 cc. lot of (3).

Sterilization: Not specified.

Use: To isolate nitrite formers from the soil and to study nitrification. Trommsdarf's reagents were used to test for the presence of nitrites.

Reference: Gowda (1924 p. 252).

INVESTIGATOR	OMELIANSKY 1899	HARVEY 1921–22	WIMMER 1904	BOULLANGER AND MASSOL 1903
Distilled water	1000.0 cc.	Amount not given	1000.0 cc.	1000.0 cc.
$(NH_4)_2SO_4$	2.0 g.	1.0 or 2.0 g.	2.0 g.	2.0 g.
NaCl	2.0 g.	2.0 g.	2.0 g.	2.0 g.
Potassium phosphate	1.0 g.			1.0 g
$MgSO_4$	0.5 g.	0.5 g.	0.5 g.	0.5 g.
$FeSO_4$	0.4 g.	0.4 g.	0.4 g.	0.4 g.
$MgCO_3$	In excess	In excess	In excess	In excess
K_2HPO_4		1.0 g.		
KH_2PO_4			1.0 g.	
KCl			0.0 or 0.28 g.	
Slag				Amount not specified

References: Winogradsky and Omeliansky (1899 p. 432), Omeliansky (1899 p. 539), Smith (1905 p. 199), Boullanger and Massol (1903 p. 493), Wimmer (1904 p. 139), Löhnis (1913 p. 97), Percival (1920 p. 144), Harvey (1921–22 p. 105), Giltner (1921 p. 375), Tanner (1919 p. 67), Cunningham (1924 p. 150).

66. Gibbs' Ammonium Sulphate Solution (modified by Gowda)

Constituents:

1.	Conductivity water	1100.0 cc.
2.	$(NH_4)_2SO_4$	1.0 g.
3.	K_2HPO_4	1.0 g.
4.	NaCl	2.0 g.
5.	$MgSO_4$	0.5 g.
6.	$Fe_2(SO_4)_3$	trace
7.	$MgCO_3$	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1000.0 cc. conductivity water.
(2) Dissolve 7 in 100.0 cc. of conductivity water.

67. Omeliansky Ammonium Sulphate Solution (Harvey)

Constituents:

1.	Water	1000.0 cc.
2.	K_2HPO_4	1.0 g.
3.	$MgSO_4$	0.5 g.
4.	$(NH_4)_2SO_4$	1.0 g.
5.	NaCl	0.06 g.
6.	Precipitated chalk	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Add 6 to (1).
(3) Distribute in flasks.

Sterilization: Not specified.

Use: Study of nitrifying bacteria.

Variants: Niklewski used the following solution to study nitrification by bacteria from stable manure. It was prepared the same as the above solution.

1.	Water	1000.0 cc.
2.	$(NH_4)_2SO_4$ (0.05%)	0.5 g.
3.	K_2HPO_4 (0.02%..)	0.2 g.
4.	$MgSO_4$ (0.01%)	0.1 g.

5. NaCl (0.01%)	0.1 g.
6. $FeCl_3$	trace
7. $CaCO_3$	

Reference: Harvey (1921–22 p. 103), Niklewski (1910 p. 410).

68. Christensen's Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2SO_4$	1.6 g.
3. K_2HPO_4	2.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Distribute in 50 cc. lots in 500.0 cc. tuberculin flasks.

(3) Add 1.0 g. of lime to each flask.

Sterilization: 20 minutes at 120°C. in autoclave.

Use: Inoculate with soil to study nitrifying bacteria.

Reference: Christensen (1913 p. 418).

69. Gage's Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2SO_4$	4.0 g.
3. KH_2PO_4	2.0 g.
4. $MgSO_4$	1.0 g.
5. $FeSO_4$	0.8 g.
6. NaCl	4.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Distribute in Erlenmeyer flasks.

Sterilization: Method not specified.

Use: Study of nitrification by soil organisms.

Reference: Gage (1910 p. 31).

70. Omeliansky's Ammonium Sulphate Solution (Löhnis-Arnd)

Constituents:

1. Distilled water	1000.0 cc.
2. $(NH_4)_2SO_4$	1.0 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4 + 7H_2O$	0.5 g.
5. NaCl	2.0 g.
6. $FeSO_4 + 7H_2O$	0.4 g.
7. K_2CO_3	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) After sterilization distribute under aseptic precautions, in 50.0 cc. lots in 300.0 cc. Erlenmeyer flasks, each containing 0.5 g. pure K_2CO_3.

Sterilization: (1) is sterilized for one hour in streaming steam. The flasks containing the K_2CO_3 are sterilized for 20 minutes under a pressure of 2 atmospheres in the autoclave.

Use: Inoculate with 5.0 g. of soil to study nitrification.

Reference: Arnd (1916 p. 561).

71. Löhnis' Ammonium Sulphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $(NH_4)_2SO_4$	1.0 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4$	0.5 g.
5. NaCl	2.0 g.
6. $FeSO_4$	0.4 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) During incubation at 20 to 25°C. allow some ordinary gas to enter the incubator.

Sterilization: Not specified.

Use: To study the assimilation of Ca_2 by *B. oligocarbophilus*.

Reference: Löhnis (1913 p. 106).

72. Stutzer's Ammonium Magnesium Phosphate Solution

Constituents:

1. Water	2000.0 cc.
2. K_2HPO_4	2.0 g.
3. NaCl	0.5 g.
4. Dry ammonium magnesium phosphate	20.0 g.
5. $FeSO_4$	0.5 g.
6. $MgCO_3$	20.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1000.0 cc. water.

(2) Dissolve 5 and 6 in 1000.0 cc. water.

(3) After sterilization mix (1) and (2) in equal amounts and distribute in sterile flasks by means of sterile pipettes.

Sterilization: Method not specified.

Use: Inoculate with a loop of soil suspension. Transfer from flask to flask, using as an enrichment medium.

Variants: Stutzer used the constituents in the following proportions:

1. Water	1000.0	cc.
2. K_2HPO_4	1.0	g.
3. NaCl	0.25	g.
4. $FeSO_4$	0.025	g.
5. Ammonium magnesium phosphate (dry)	20.0	g.
6. $MgCO_3$	20.0	g.

The medium was prepared by mixing 5 and 6 in a mortar and adding a teaspoonful or more of the mixture to flat bottomed flasks. Then add 50.0 to 100.0 cc. of a solution of 2, 3 and 4 in 1 to each flask. Sterilize the flasks. Method not given.

Reference: Stutzer (1901 p. 173).

SUBGROUP I-B. SECTION 5

Inorganic liquid media of known composition, nitrogen supplied as ammonium salts, complete nutrient solutions, but not used primarily for organisms oxidizing ammonia to nitrites.

A few media have been described containing ammonium salts but no organic carbon for the cultivation of microörganisms which do not secure their growth energy by oxidation of ammonia. These media are of two general classes, those used with sulphur or its compounds for the cultivation of the bacteria oxidizing sulphur, and those for other types of organisms, including the algae. The various media may be differentiated as follows:

A_1. Primarily for organisms oxidizing sulphur or its compounds.
- Waksman's Ammonia and Sulphur Solution no. 2........ 73
- Waksman's Ammonia and Sulphur Solution........ 74
- Waksman's Sulphide Solution........ 75
- Beijerinck's Thiosulphate Solution........ 76

A_2. Not primarily for organisms oxidizing sulphur.

B_1. Ammonia supplied as ammonium chloride.
- Kaserer's Hydrogen Ammonium Chloride Solution........ 77
- Beijerinck and van Delden's Ammonium Chloride Solution........ 78

B_2. Ammonia supplied as ammonium nitrate.
- Richter's Ammonium Nitrate Solution........ 79

B_3. Ammonia supplied as ammonium sulphate.
- Lieske's Ammonium Sulphate Solution........ 80
- Gottheil's Ammonium Sulphate Solution. (No. XI)........ 8.
- Gosling's Ammonium Sulphate Solution........ 82
- Beijerinck and van Delden's Ammonium Sulphate Solution........ 83

B_4. Ammonia supplied as a salt of phosphoric acid.
- Beijerinck and van Delden's Manganese Ammonium Phosphate Solution........ 84
- Beijerinck and van Delden's Ammonium Phosphate Solution........ 85

73. Waksman's Ammonia and Sulphur Solution No. 2

Constituents:

1. Distilled water	1000.0	cc.
2. $(NH_4)_2SO_4$	0.2	g.
3. $MgSO_4 \cdot 7H_2O$	0.5	g.
4. KH_2PO_4	1.0	g.
5. $Ca_3(PO_4)_2$ (re-precipitated)	2.5	g.
6. Sulfur	10.0	g.
7. H_3PO_4		

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Weigh sulfur and $Ca_3(PO_4)_2$ separately into the individual flasks into which the medium is distributed (100.0 cc. portions are usually placed in 250 cc. flasks).

(3) Adjust to pH = 3.0 by the addition of M/1 H_3PO_4.

Sterilization: Sterilize in flowing steam for 30 minutes on 3 successive days.

Use: Isolation and cultivation of *Thiobacillus thiooxidans.*

Reference: Waksman (1922 p. 606).

74. Waksman's Ammonia and Sulphur Solution

Constituents:

1. Distilled water	1000.0	cc.
2. $(NH_4)_2SO_4$	0.2	g.
3. $MgSO_4 \cdot 7H_2O$	0.5	g.
4. KH_2PO_4	3.0	g.
5. $CaCl_2$	0.25	g.
6. Sulphur (powdered)	10.0	g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Weigh out the sulphur separately in the individual flasks into which the

(1) is distributed (100 cc. in 250 cc. flasks usually).

Sterilization: Sterilize in flowing steam for 30 minutes on 3 consecutive days.

Use: Isolation and cultivation of *Thiobacillus thiooxidans*. The organisms can grow at a pH = 1.0 with a maximum at pH = 3.0 to 4.0.

Variants: The author added a trace of $FeSO_4$ and the following combinations of potassium salts of phosphoric acid to the above solution containing no potassium phosphate.

(a) KH_2PO_4 3.0 g. pH = 4.2

(b) $\left\{\begin{array}{l}KH_2PO_4\ 1.5\ g.\\ K_2HPO_4\ 1.5\ g.\end{array}\right\}$ pH = 5.4

(c) K_2HPO_4 3.0 g. pH = 6.0

(d) $\left\{\begin{array}{l}K_2HPO_4\ 3.0\ g.\\ CaCO_3\ \ 10.0\ g.\end{array}\right\}$ pH = 8.0

At the various pH values the author found starting with acid medium some *Th. thiooxidans* give final pH = 1.2. Soil bacteria produce no acid at pH = 4.2 but change pH = 8 to pH = 7.6.

Reference: Waksman (1922 pp. 606, 609 to 613).

75. Waksman's Sulphide Solution

Constituents:

1. Water	1000.0	cc.
2. K_2S	5.0	g.
3. NH_4Cl	0.1	g.
4. $MgCl_2$	0.1	g.
5. Na_2HPO_4 or K_2HPO_4	0.2	g.
6. $NaHCO_3$	1.0	g.
7. $CaCO_3$	10.0	g.
or $CaCl_2$	0.25	g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in part of 1.

(2) Dissolve 6 and 7 in rest of 1.

(3) Add (1) to (2) by means of sterile pipettes after sterilization.

(4) pH = 7.5.

Sterilization: Autoclave at 15 pounds pressure for 15 minutes.

Use: To show oxidation of K_2S by *Thiobacillus thiooxidans*.

Reference: Waksman (1922 p. 609–15).

76. Beijerinck's Thiosulphate Solution

Constituents:

1. Water	100.0	cc.
2. $Na_2S_2O_3 \cdot 5H_2O$	0.5	g.
3. $NaHCO_3$	0.1	g.
4. K_2HPO_4	0.02	g.
5. NH_4CL	0.01	g.
6. $MgCl_2$	0.01	g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Adjustment of reaction not specified.

(3) Inoculate with a little ditch or canal water or ditch mud. Incubate at 28 to 30°C.

Sterilization: None required.

Use: Enrichment and isolation of organisms utilizing carbonic acid, *Thiobacillus thioparus*. Two or three days after inoculation the surface is covered with a layer of free sulphur filled with bacteria.

Variants:

(a) When Beijerinck used an inoculum from sea water, 3.0% NaCl was added to the solution.

(b) Waksman used the above solution with either 0.02 g. Na_2HPO_4 or K_2HPO_4 and added one of the following combinations:

(1) KH_2PO_4 3.0 g. pH = 5.4

(2) $NaHCO_3$ 1.0 g. pH = 8.8

(3) $\left\{\begin{array}{l}NaHCO_3\\ CaCO_3\end{array}\right.\begin{array}{l}1.0\ g.\\ 10.0\ g.\end{array}$ pH = 9.4

He found that *Thiobacillus thiooxidans* produced a pH = 1.4 in variant (b) (1).

(c) Trautwein substituted Na_2HPO_4 for K_2HPO_4 in the original solution and used the solution to grow Thionic acid bacteria (Omeliansky).

Reference: Beijerinck (1903 p. 595), Waksman (1922 p. 609), Trautwein (1921 p. 518).

77. Kaserer's Hydrogen Ammonium Chloride Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4 (0.05%)	0.5 g.
3. $MgSO_4$ (0.02%)	0.2 g.
4. NH_4Cl (0.1%)	1.0 g.
5. $NaHCO_3$ (0.05%)	0.5 g.
6. Iron chloride	trace
7. Hydrogen	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Flask.

(3) Following sterilization, if the solution is sterilized, inoculate the solution with soil.

(4) Prepare hydrogen gas from c.p. H_2SO_4 and zinc.
(5) Pass hydrogen and carbonic acid into the flask.

Sterilization: Not specified.

Use: To show oxidation by soil forms, *Bacillus oligocarbophilus* and *Bacillus methylicus*. After 5 days the amount of hydrogen was less in the flask inoculated with soil than in the control flask.

Variants: Lantzsch using the same solution found that *Actinomyces* (*Bacillus oligocarbophilus* oxidized hydrogen in a non-sterilized medium, but failed in a sterilized medium.

Reference: Kaserer (1906 p. 686), Lantzsch (1922 p. 316).

78. Beijerinck and van Delden's Ammonium Chloride Solution

Constituents:

1. Distilled water	1000.0 cc.
2. NH_4Cl	0.1 to 1.0 g.
3. Potassium phosphate	0.2 g.
4. Beijerinck and van Delden's basic salt solution (see p. 12)	10 drops.

Preparation:
(1) Dissolve 2 and 3 in 1.
(2) Add 1.0 drop of Beijerinck and van Delden's basic salt solution (see page 12) to each 100.0 cc. of (1).

Sterilization: Not specified.

Use: Cultivation of *Bacillus oligocarbophilus*.

Reference: Beijerinck and van Delden (1903 p. 36).

79. Richter's Ammonium Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. NH_4NO_3	3.0 g.
3. $MgSO_4$	1.0 g.
4. KH_2PO_4, $FeSO_4$ and $ZnSO_4$	1.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Growth of *Aspergillus niger*.

Reference: Richter (1910 p. 619).

80. Lieske's Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. Magnesium bicarbonate	
3. $NaHCO_3$ (0.001%)	0.01 g.
4. $(NH_4)_2SO_4$ (0.001%)	0.01 g.
5. Potassium phosphate	trace
6. $MgSO_4$	

Preparation:
(1) Prepare Magnesium bicarbonate by adding pure carbonic acid to a suspension of Magnesium carbonate.
(2) Filter.
(3) Dilute the filtrate 1 to 10 with water.
(4) Dissolve 3, 4, 5 and 6 in (3).

Sterilization: Not specified.

Use: Cultivation of *Leptothrix ochracea*.

Reference: Lieske (1919 p. 422).

81. Gottheil's Ammonium Sulphate Solution (No. XI)

Constituents:

1. Distilled water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.3 g.
5. NaCl	0.1 g.
6. Iron	trace
7. $(NH_4)_2SO_4$	0.25 mg.
8. Na_2CO_3	0.5 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: Cultivation of organisms found in the soil on roots and rhizomes.

Reference: Gottheil (1901 p. 432).

82. Gosling's Ammonium Sulphate Solution

Constituents:

1. Distilled water	500.0 cc.
2. Na_2CO_3 (dehydrated)	2.3 g.
3. $CaCO_3$	0.25 g.
4. K_2CO_3	0.08 g.
5. $FeSO_4$	0.03 g.
6. $(NH_4)_2SO_4$	0.005 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To determine sulphate reduction.

Reference: Gosling (1904 p. 391).

83. Beijerinck and van Delden's Ammonium Sulphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $(NH_4)_2SO_4$	1.0 g.
3. Potassium phosphate	0.2 g.

4. Beijerinck and van Delden's mineral solution (see p. 12).	

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add 1.0 drop of 4 to each 100.0 cc. of (1).

Sterilization: Not specified.

Use: Growth of *Bacillus oligocarbophilus.*

Reference: Beijerinck and van Delden (1903 p. 36).

84. Beijerinck and van Delden's Manganese Ammonium Phosphate Solution

Constituents:

1. Distilled water...........	1000.0 cc.
2. $(NH_4)_2HPO_4$.............	0.2 g.
3. Na_2CO_3..................	1.0 g.
4. Beijerinck and van Delden's solution (see p. 12)..	20 drops.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add 2.0 drops of 4 to each 100.0 cc. of (1).

Sterilization: Not specified.

Use: *Bacillus oligocarbophilus* grows as a snow white scum on the surface of the medium.

Reference: Beijerinck and van Delden (1903 p. 41).

85. Beijerinck and van Delden's Ammonium Phosphate Solution

Constituents:

1. Distilled water...............	100.0 cc.
2. K_2HPO_4....................	0.02 g.
3. $(NH_4)_2HPO_4$...............	0.02 g.
4. Na_2CO_3....................	0.1 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Reaction to be slightly alkaline.

Sterilization: Not specified.

Use: *Bacillus oligocarbophilus* grows as a thin white scum on the medium.

Reference: Beijerinck and van Delden (1903 p. 41).

SUBGROUP I-B. SECTION 6

Inorganic liquid media or basal solutions of known composition; nitrogen supplied as nitrites.

The several liquid media which have been published for the growth of organisms oxidizing nitrites may be differentiated as follows:

A_1. Employed primarily for organisms oxidizing nitrites to nitrates.
B_1. Calcium carbonate added.
Hewlett's Nitrite Solution (Johnson). 86
Cunningham's Nitrite Solution....... 87
B_2. Calcium carbonate not added.
C_1. Magnesium carbonate added.
Omeliansky's Nitrite Solution........ 88
C_2. Magnesium carbonate not added.
D_1. Magnesium sulphate added.
Winogradsky and Omeliansky's Sodium Nitrite Solution............... 89
Winogradsky's Basal Sodium Nitrite Solution.......................... 90
D_2. Magnesium sulphate not added.
Gärtner's Sodium Nitrite Solution... 91
Wimmer's Sodium Nitrite Solution... 92
Bijerinck and van Delden's Basal Nitrite Solution.................... 93
A_2. Not employed primarily for organisms oxidizing nitrites to nitrates.
Gottheil's Nitrite Solution No. VIII. 94
Bijerinck and van Delden's Potassium Nitrite Solution A................. 95

86. Hewlett's Nitrite Solution (Johnson)

Constituents:

1. Distilled water.............	1000.0 cc.
2. KNO_2.....................	0.3 g.
3. Potassium phosphate.......	0.1 g.
4. $MgSO_4$....................	0.05 g.
5. $CaCO_3$....................	5.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Study of nitrate production. *Bacillus megatherium* produced no nitrates in this solution.

Reference: Johnson (1912 p. 219).

87. Cunningham's Nitrite Solution

Constituents:

1. Distilled water.............	1000.0 cc.
2. $NaNO_2$....................	1.0 g.
3. K_2HPO_4..................	0.5 g.
4. $MgSO_4$....................	0.3 g.
5. NaCl......................	0.5 g.
6. Na_2CO_3..................	0.2 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Distribute in 10.0 cc. quantities in 50.0 cc. Erlenmeyer flasks.

(3) Add a small quantity of sterile $CaCO_3$ to each tube of sterile (2).

Sterilization: Sterilize (2) and the $CaCO_3$ into autoclave.

Use: To study oxidation of nitrites and nitrates.

Reference: Cunningham (1924 p. 151).

88. Omelianski's Nitrite Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $NaNO_2$ (Merck)	1.0 g.
3. Na_2CO_3 (ustum)	1.0 g.
4. Potassium phosphate	0.5 g.
5. NaCl	0.5 g.
6. $FeSO_4$	0.4 g.
7. $MgSO_4$	0.3 g.
8. $MgCO_3$	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Flask in flat bottomed flasks in 50.0 cc. lots.

(3) Add 0.5 g. $MgCO_3$ to each flask.

Sterilization: Not specified.

Use: Isolation of nitrate forming organisms. The author found that repeated cultivation on this medium yielded a pure culture.

Variants: Boullanger and Massol used the above solution with the addition of slag.

Reference: Omeliansky (1899 p. 548), Boullanger and Massol (1903 p. 494).

89. Winogradsky and Omeliansky's Sodium Nitrite Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $NaNO_2$	1.0 g.
3. Potassium phosphate	0.5 g.
4. $MgSO_4$	0.3 g.
5. NaCl	0.5 g.
6. Na_2CO_3	1.0 g.
7. $FeSO_4$	0.4 g.

Preparation:

(1) Distill water with permanganate.

(2) Dissolve 2, 3, 4 and 5 in (1).

(3) Distribute in 50.0 cc. lots.

(4) Add 0.0, 0.1, 0.2, 0.4, 0.5 or 0.6 g. of Na_2CO_3 to each flask (1.0% gives best results).

(5) Iron may be added.

Sterilization: Not specified.

Uses: To study nitrate production from nitrites. The author found that the addition of 0.1% Na_2CO_3 gave the best results. The addition of $FeSO_4$ had no effect on nitrate production.

Added nutrients: The authors added one of the following materials to determine the effect of organic material on nitrification.

glycerol 0.1, 0.2, 0.4, 0.8, 1.0, 1.4 or 2.0%
glucose 0.05, 0.1, 0.2, 0.4, 0.8, 1.0 or 2.0%
urea 0.1, 1.0 or 1.6%
asparagin 0.1, 0.6, 0.8, 1.0 or 2.0%
hay infusion 4.0, 8.0, 16.0 or 32.0%
leaf infusion 4.0, 8.0, 16.0 or 32.0%
dirt infusion 4.0, 8.0, 16.0 or 32.0%
meat infusion 2.0, 4.0, 8.0 or 10.0%
urine 2.0, 4.0, 10.0 or 20.0%
$(NH_4)_2SO_4$ (1.0% solution) 0.2, 1.0, 1.6, 2.0, 3.0, 4.0 or 6.0%
sodium acetate 1.0, 2.0, 3.0, 4.0 or 6.0%
sodium butyrate 0.1, 1.0, 2.0, 3.0 or 4.0%

Generally organic material slows up oxidation unless it be present in very small quantities. The more organic materials present the slower the oxidation.

Variants:

(a) Wimmer used the above solution specifying 0.5 g. KH_2PO_4 and suggesting the addition of 0.28 g. KCl.

(b) Harvey used this solution employing 0.5 g. K_2HPO_4 and 0.3 g. anhydrous Na_2CO_3 instead of 1.0 g.

(c) Gibbs (Gowda) used 0.5 g. K_2HPO_4 and a trace of $Fe_2(SO_4)_3$ instead of 0.4 g. $FeSO_4$.

Reference: Winogradsky and Omeliansky (1899 p. 333), Wimmer (1904 p. 140), Smith (1905 p. 199), Harvey (1921–22 p. 106), Gowda (1924 p. 258), Percival (1920 p. 149).

90. Winogradsky's Basal Sodium Nitrite Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $NaNO_2$ (Merck)	1.0 g.
3. Potassium phosphate	0.5 g.
4. $MgSO_4$	0.3 g.
5. Na_2CO_3	0.5 g.
6. NaCl	0.5 g.

Preparation:

(1) Distill water two times with "chamäleon."

(2) Dissolve 2, 3, 4 and 5 in 1. The Na_2CO_3 should be water free, heated to a weak glowing.

(3) Adjustment of reaction not specified.
(4) Flask in 50.0 cc. lots.

Sterilization: Sterilize in the autoclave.

Use: To study nitrification.

Added nutrients and variants: The author added glucose, urea, asparagin or peptone to the solution to determine the effect of organic materials on nitrification. Distribute in 50.0 cc. lots and add:

(a) 0.2, 0.4, 0.8, 1.0, 1.2, 1.6, 2.0, 2.4 or 4.0 g. glucose.
(b) 0.05, 0.1, 0.2, 0.4, 0.8, 1.2 or 1.6 g. peptone.
(c) 0.1, 0.2, 0.4 or 0.8 g. urea.
(d) 0.1, 0.2 or 0.4 g. asparagin.
(e) 5.0 cc. of bouillon.

The presence of organic materials generally tends to slow up the oxidation. Peptone in small amounts tends to hasten oxidation. Same is true of urea. Asparagin and glucose even in small amounts slow up the oxidation.

Variants: The modifications shown in the table have been described. Their preparation is like the original solution. Stutzer and Hartleb bubbled air containing CO_2 thru their solution. Carbonates or CO_2 were essential for nitrate production.

AUTHOR	WINOGRADSKY AND OMELIANSKY	STUTZER	STUTZER AND HARTLEB	GILTNER AND LÖHNIS
Distilled water	1000.0 cc.	1000.0 cc.	1000.0 cc.	1000.0 cc.
$NaNO_2$	1.0 g.	2.0 g.	25.0 cc. 2.0% solution	1.0 g.
Potassium phosphate	0.5 g.		1.0 g.	
$MgSO_4$	0.3 g.	0.3 g.	0.5 g.	0.3 g.
NaCl	0.5 g.	0.5 g.	1.0 g.	0.5 g.
Na_2CO_3	1.0 g.			0.3 g.
K_2HPO_4		1.0 g.		1.0 g.
K_2CO_3		0.5 g.		
KCl			0.1 g.	

References: Winogradsky (1896 p. 423), Stutzer and Hartleb (1897 p. 244), Winogradsky and Omeliansky (1899 p. 378), Stutzer (1901 p. 169), Löhnis (1913 p. 97) Giltner (1921 p. 375).

91. Gärtner's Sodium Nitrite Solution

Constituents:

1. Water	1000.0 cc.
2. Potassium phosphate	0.5 g.
3. K_2CO_3	5.0 g.
4. $NaNO_2$	2.0 g.

Preparation:

(1) Dissolve 2, 3, and 4 in 1.
(2) Distribute in 30 cc. lots in flasks.

Sterilization: Not specified.

Use: To study the oxidation of nitrite by fungus. Nitrite was found to be still present after four weeks.

Reference: Gärtner (1898 p. 4).

92. Wimmer's Sodium Nitrite Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Natrium nitrosum puriss ($NaNO_2$)	1.0, 2.0 or 50.0 g.

Preparation: (1) Dissolve 2 in 1.

Sterilization: Not specified.

Use: To study nitrification by soil bacteria.

Reference: Wimmer (1904 p. 140).

93. Beijerinck and van Delden's Basal Nitrite Solution

Constituents:

1. Water	1000.0 cc.
2. KNO_2	0.5 g.
3. K_2HPO_4	0.5 g.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Add one of the added nutrients to (1).

Sterilization: Not specified.

Use: To study denitrification by *B. subtilis*, *B. mesentericus* and *Azo. chroomcoccum*. *B. subtilis* gave no ammonia test. *B. mesentericus* gave ammonia using mannitol as a carbon source. Chroococcum gave ammonia using either mannitol or malate as a carbon source.

Added nutrients: The authors used either 20.0 g. of mannitol or 20.0 g. calcium malate as a carbon source.

Reference: Beijerinck and van Delden (1902 p. 41).

94. Gottheil's Nitrite Solution No. VIII

Constituents.

1. Distilled water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.3 g.
5. NaCl	0.1 g.
6. Iron	trace
7. KNO_2	0.05 g.
8. Soda	0.05 g.

Preparation: Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: Cultivation of bacteria found in soil on roots and rhizomes.

Reference: Gottheil (1901 p. 432).

95. Beijerinck and van Delden's Potassium Nitrite Solution A

Constituents:

1. Water (tap)	100.0 cc.
2. KCl	0.01 g.
3. K_2HPO_4	0.02 g.
4. KNO_2	0.01 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) The reaction is slightly alkaline.

Sterilization: Not given.

Use: *Bacillus oligocarbophilus* grows in a dry thin snow white scum on the surface of the medium.

Reference: Beijerinck and van Delden (1903 p. 41).

SUBGROUP I-B. SECTION 7

Inorganic basal solutions of known compositions; nitrogen supplied as nitrates; incomplete solutions requiring the addition of other nutrients.

A_1. Salts of monovalent cations only added.
  Beijerinck's Basal Nitrate Solution. 96
  Stoklasa and Vitek's Basal Nitrate Solution 97
A_2. Salts of mono and divalent cations added.
  Czapek's Basal Nitrate Solution (Waksman) 98
  Giltay-Aberson's Basal Nitrate Solution (Stoklasa) 99
  Henneberg's Basal Nitrate Solution. 100
A_3. Salts of mono, di and tri valent cations added.
B_1. Calcium salts added.
  Stoklasa and Vitek's Basic Nitrate Salt Solution 101
  Harvey's Basal Nitrate Solution 102
B_2. Calcium salts not added.
  Beijerinck and van Delden's Nitrate Solutions, B, E, F, H, N 103
  Kita's Basal Nitrate Solution 104
  Stoklasa's Basal Nitrate Solution 105
  Lantzsch's Basal Nitrate Solution 106

96. Beijerinck's Basal Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. KNO_3	1.0 g.
3. K_2HPO_4	0.2 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add one of the materials listed under added nutrients.

Sterilization: Not specified.

Use: To study denitrification by *Azotobacter chroococcum.*

Added nutrients and variants:

(a) The author added 20.0 to 100.0 g. mannitol.

(b) Beijerinck and van Delden used 0.2 g. KNO_3 in the basic solution without additional nutrients for the cultivation of *Bacillus oligocarbophilus.*

(c) Beijerinck and van Delden used 0.2 g. KNO_3 and 0.2 g. Na_2HPO_4 in the basic solution and added 0.2 g. KCl for the cultivation of *Bacillus oligocarbophilus.*

(d) Beijerinck and van Delden used 0.1 g. KNO_3 and 0.1 g. K_2HPO_4 in the basic solution and added 0.1 g. Na_2CO_3 for the cultivation of *Bacillus oligocarbophilus.*

(e) Beijerinck and Minkman used 1.0% KNO_3 and 0.05% K_2HPO_4 in the basic solution and added 2.0% mannitol, glycerol, sodium acetate or sodium propionate to study denitrification.

(f) Beijerinck and van Delden used 0.5 g. KNO_3 and 0.5 g. K_2HPO_4 in the basic solution and added 2.0 g. of calcium malate or mannitol to study denitrification.

References: Beijerinck (1901 p. 575), Beijerinck and van Delden (1902 p. 41), (1903 p. 41), Beijerinck and Minkman (1910 pp. 35, 36).

97. Stoklasa and Vitek's Basal Nitrate Solution

Constituents:

1.	Water	1000.0 cc.
2.	$NaNO_3$	8.5 g.

Preparation:

(1) Dissolve 2 in 1.

(2) Add a suitable carbon source.

Sterilization: Not specified.

Use: To study nitrate reduction by *Bact. Hartlebi* and other bacteria.

Added nutrients: The author suggested the addition of the following carbon sources:

xylose	15.0 g.
glucose	18.0 g.
sucrose	34.2 g.

Reference: Stoklasa and Vitek (1905 p. 205).

98. Czapek's Basal Nitrate Solution (Waksman)

Constituents:

1.	Water	1000.0 cc.
2.	K_2HPO_4	1.0 g.
3.	$MgSO_4$	0.5 g.
4.	KCl	0.5 g.
5.	$FeSO_4$	0.01 g.
6.	$NaNO_3$	2.0 g.

Preparation:

(1) Prepare a solution of 1, 2, 3, 4, 5 and 6 using chemically pure materials.

(2) Add 20.0 g. of one of the test materials.

Sterilization: Autoclave at 15 pounds pressure for 15 minutes.

Use: To study the metabolism of actinomycetes. The author found that the Actinomycetes utilized the materials in order listed. Starch, glucose, lactose, maltose, glycerin, sucrose, cellulose and then organic acids.

Added nutrients: The author used 20.0 g. of one of the following carbon sources:

arabinose
glycerol
lactose
sucrose
starch
sodium salts of:
  acetic acid
  malic acid
maltose
mannitol
inulin
glucose
sodium salts of:
  tartaric acid
  oxalic acid
  lactic acid
cellulose in form of Whitman filter paper
  1.0 cc. of 0.5% cellulose suspension.

Reference: Waksman (1919 p. 317).

99. Giltay-Aberson's Basal Nitrate Solution (Stoklasa)

Constituents:

1.	Water	1000.0 cc.
2.	$NaNO_3$	2.0 g.
3.	$MgSO_4$	2.0 g.
4.	K_2HPO_4	2.0 g.
5.	NaCl	0.2 g.
6.	Iron chloride less than	0.2 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Adjust to slightly alkaline reaction by adding Na_2CO_3.

(3) Add one of the combinations given in added nutrients.

Sterilization: Not specified.

Use: To study denitrification and influence of carbohydrates on denitrification by soil forms.

Added nutrients: The author added one of the following combinations:

(a) Citric acid 5.0 g. and glucose 2.0 g.

(b) Glucose 2.0 g.

(c) Glucose 2.0 g. with stable manure.

(d) Glucose 2.0 g. and citric acid 5.0 g. with stable manure.

(e) Glucose 2.0 g. with Chilisaltpeter.

(f) Glucose 2.0 g. and citric acid 5.0 g. with Chilisaltpeter.

(g) Chilisaltpeter, peat, or soil rich in humus.

Reference: Stoklasa (1907 p. 28).

100. Henneberg's Basal Nitrate Solution

Constituents:

1.	Water	1000.0 cc.
2.	$MgSO_4$	1.0 g. or 2.0 g.
3.	KNO_3	2.0 g. or 3.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add one of the test materials to (1).

Sterilization: Not specified.

Use: To determine the fermentation of acetic acid bacteria, *B. oxydans*, *B. acetosum*, *B. aceti*, *B. Kützingianum*, *B. Pasteurianum*, *B. acetigenum*. *B. oxy-*

dans ferments arabinose, levulose, dextrose, galactose, maltose, dextrin, ethyl and propyl alcohol, erythritol. *B. acetosum* ferments arabinose, dextrose, galactose, ethyl and propyl alcohol. *B. aceti*, *B. Kützingianum*, *B. Pasteurianum* and *B. acetigenum* ferment dextrose and ethyl and methyl alcohol.

Added nutrients: The author added the following carbon sources:

arabinose
levulose
glucose
galactose
sarobose
sucrose
maltose
lactose
dextrin
starch
glycogen
inulin
methyl alcohol
ethyl alcohol
propyl alcohol
amyl alcohol
glycerol
erythritol
mannitol
dulcitol
melampyrit
quercitol

Reference: Henneberg (1898 p. 19).

101. Stoklasa and Vitek's Basal Nitrate Salt Solution

Constituents:

1. Water	1000.0 cc.
2. $NaNO_3$	2.0 g.
3. K_2HPO_4	1.25 g.
4. K_2SO_4	0.2 g.
5. $CaCl_2$	0.05 g.
6. $MgCl_2$	0.05 g.
7. Na_2CO_3	0.1 g.
8. $FePO_4$	0.05 g.

Preparation:

(1) Prepare solutions as indicated in the table by dissolving the salts in water.
(2) Add 2.0 or 2.5 g. of test material.

Sterilization: Not specified.

Use: To study reduction of nitrates by denitrifying organisms.

Added nutrients: The author added a variety of carbohydrates (2.0 or 2.5 g.) or 2.0 g. of organic acids. Neutralize the acids with Na_2CO_3.

butyric acid
succinic acid
lactic acid
citric acid
malic acid
tartaric acid
asparagin
glucose
levulose
galactose
arabinose
xylose
saccharose
lactose

Variants: The authors substituted 0.25 g. Na_2HPO_4 for 1.25 g. K_2HPO_4.

Reference: Stoklasa and Vitek (1905 p. 104) (1901 p. 262).

102. Harvey's Basal Nitrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $NaNO_3$	1.0 g.
3. NaCl	0.02 g.
4. $MgSO_4$	0.02 g.
5. K_2HPO_4	0.5 g.
6. $FeCl_3$	trace

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Add one of the added nutrients.

Sterilization: Not specified.

Use: Study of nitrate reduction.

Added nutrients: The author added 10.0 g. glucose or 10.0 g. glycerol.

Variants: The author used the following solution:

1. Distilled water	3000.0 cc.
2. KNO_3	7.0 g.
3. K_2HPO_4	1.5 g.
4. $MgSO_4$	1.5 g.
5. NaCl	1.5 g.
6. $CaSO_4$	5.0 g.
7. $FeCl_3$ solution	few drops

Reference: Harvey (1921–22 p. 104).

103. Beijerinck and van Delden's Nitrate Solution B, E, F, H, N

Constituents:

Solution B:

1. Distilled water	1000.0 cc.
2. KCl	0.2 g.
3. KNO_3	1.0 g.
4. K_2HPO_4	0.2 g.

Solution E:

1. Distilled water	1000.0 cc.
2. KCl	0.1 g.
3. KNO_3	1.0 g.
4. K_2HPO_4	0.2 g.

Solution F:

1. Distilled water	1000.0 cc.
2. KNO_3	0.1 g.
3. Na_2HPO_4	0.2 g.

Solution H:

1. Distilled water	1000.0 cc.
2. KCl	0.2 g.
3. KNO_3	0.2 g.
4. Na_2HPO_4	0.2 g.

Solution N:

1. Distilled water	1000.0 cc.
2. KNO_3	0.1 to 1.0 g.
3. K_2HPO_4	0.1 g.

Preparation:

(1) Dissolve the salts in water as indicated in the table.

(2) Add one drop of Beijerinck and van Delden's mineral solution (see Med. 25) to each 100.0 cc. of (1).

Sterilization: Not specified.

Use: *Bacillus oligocarbophilus* grows as a dry thin snow white scum on the surface of the medium.

Reference: Beijerinck and van Delden (1903 p. 41).

104. Kita's Basal Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4$	0.25 g.
3. KH_2PO_4	5.0 g.
4. $FeCl_3$ solution	few drops
5. KNO_3	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add 5.0% of any carbohydrate.

(3) Adjustment of reaction not specified.

Sterilization: Not specified.

Use: Cultivation of molds, *Aspergillus albus*, *Aspergillus Okazaki*, *Aspergillus candidus*, *Aspergillus tamarii*, *Pseudorhizopus*, *Aspergillus glaucus*.

Added nutrients: The author did not specify the carbohydrates used.

Reference: Kita (1913 p. 434).

105. Stoklasa's Basal Nitrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. K_2SO_4	1.0 g.
3. $MgCl_2$	0.5 g.
4. Iron sulphate	0.1 g.
5. $NaNO_3$	
6. Monodiferric phosphate $Fe_2O_3(P_2O_5)_3$ or monodialuminum phosphate $Al_2O_3P_2O_5$	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and one of 6 in 1.

(2) Add 2.0 g. of nitrogen in the form of $NaNO_3$ and 20.0 g. of test material to (1).

(3) Distribute in Fernbach flasks.

Sterilization: Sterilize in the autoclave.

Use: To study the cycle of the phosphate ion in the soil. *Bact. fluorescens liquefaciens* and *Bact. Hartlebi* were the organisms used.

Added nutrients: The author added 20.0 g. of any carbohydrate.

Reference: Stoklasa (1911 p. 479).

106. Lantzsch's Basal Nitrate Solution

Constituents:

1. Water (tap)	100.0 cc.
2. K_2HPO_4	(0.01%) = 0.01 g.
3. $NaNO_3$	(0.01%) = 0.01 g.
4. $MgSO_4$	trace
5. $FeCl_3$	trace

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 100.0 g. tap water or in 20 parts tap water + 80 parts distilled water.

(2) Place in an atmosphere of one of the test materials.

Sterilization: Not specified.

Use: To study utilization of gaseous carbon compounds.

Added nutrients: The author prepared the various atmospheres of test materials as follows:

(a) Decompose 0.6 g. lead formate with H_2SO_4, in a 2500.0 cc. flask.

(b) 2.0 cc. of a 4.0% formalin solution in a 2000.0 cc. flask.

(c) 0.125 g. CH_3COOH in 2500 cc. flask.

(d) 0.1 g. acetone in a 2000.0 cc. flask.

(e) 0.2 cc. butyric acid in 5 liter flask.

(f) 0.12 cc. of benzol or xylol in 2000.0 cc. flask.

Reference: Lantzsch (1922 p. 310).

SUBGROUP I-B. SECTION 8

Inorganic liquid media of known composition; nitrogen supplied as nitrates; complete nutrient solutions.

A_1. Containing oxidizable sulphur.
B_1. Sulphur added as elementary sulphur.
  Beijerinck's Sulphur Nitrate Solution 107
B_2. Sulphur added as thiosulphate.
  Lieske's Thiosulphate Nitrate Solution (Gehring) 108
  Trautwein's Thiosulphate Nitrate Solution 109
  Nathansohn's Thiosulphate Nitrate Solution (Trautwein) 110
A_2. Not containing oxidizable sulphur.
  Beijerinck's Ammonium Nitrate Solution 111
  Nabokich and Leedeff's Hydrogen Nitrate Solution 112
  Bokorny's Calcium Nitrate Solution 113

107. Beijerinck's Sulphur Nitrate Solution

Constituents:

1. Ditch water or distilled water	100.0 cc.
2. Sulphur (powder)	10.0 g.
3. KNO_3	0.05 g.
4. Na_2CO_3	0.02 g.
5. $CaCO_3$	2.0 g.
6. K_2HPO_4	0.02 g.
7. $MgCl_2$	0.0 or 0.01 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Completely fill a well stoppered flask with (1).

Sterilization: Not specified.

Use: To study denitrification with sulphur oxidation by *Thiobacillus denitrificans*. The author found that the nitrate was reduced, and the sulphur oxidized to sulphate, with the liberation of nitrogen gas.

Reference: Beijerinck (1903–4 p. 597).

108. Lieske's Thiosulphate Nitrate Solution (Gehring)

Constituents:

1. Distilled water	100.0 cc.
2. $Na_2S_2O_3$	0.5 g.
3. KNO_3	0.5 g.
4. $NaHCO_3$	0.1 g.
5. K_2HPO_4	0.02 g.
6. $MgCl_2$	0.01 g.
7. Fe_2Cl_6	trace
8. $CaCl_2$	trace

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

(2) Distribute in test tubes 45 cm. high.

Sterilization: Not specified.

Use: Isolation and cultivation of denitrifying thiosulphate bacteria, *Thiobacillus denitrificans*.

Reference: Gehring (1914 p. 405), Trautwein (1921 p. 518).

109. Trautwein's Thiosulphate Nitrate Solution

Constituents:

1. Distilled water	100.0 cc.
2. $Na_2S_2O_3$	0.5 g.
3. KNO_3	0.5 g.
4. Na_2CO_3	0.1 g.
5. K_2HPO_4	0.02 g.
6. $MgCl_2$	0.01 g.
7. KCl	trace
8. Iron chloride	trace

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

(2) Distribute in tall glass cylinders.

(3) Inoculate with waste water filtrate.

Sterilization: Not specified.

Use: Enrichment and isolation of thionic bacteria (Omeliansky). A slight opalescence is formed after 6 days. After a milky turbid culture results, a snow white membrane is formed on the surface.

Variants: The author suggested the following solution:

1. Distilled water	1000.0 cc.
2. $MgCl_2$	0.01 g.
3. KNO_3	0.1 g.
4. Na_2HPO_4	0.02 g.
5. $Na_2S_2O_3$	0.2 g.
6. $NaHCO_3$	0.1 g.

Reference: Trautwein (1920 p. 515) (1921 p. 515).

110. Nathansohn's Thiosulphate Nitrate Solution (Trautwein)

Constituents:

1. Distilled water	100.0 cc.
2. NaCl	3.0 g.
3. $MgCl_2$	0.25 g.
4. KNO_3	0.1 g.
5. Na_2HPO_4	0.05 g.
6. $Na_2S_2O_3$	0.2 or 0.1 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of thionic bacteria (Omeliansky).

Reference: Trautwein (1921 p. 518).

111. Beijerinck's Ammonium Nitrate Solution

Constituents:

1. Water (tap)	100.0 cc.
2. NH_4NO_3	0.2 g.
3. Potassium bi-phosphate	0.05 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: Cultivation of gonidia of algae.

Variants: Beijerinck used 0.02 g. NH_4NO_3 and 0.02 g. K_2HPO_4 in 100.0 cc. of tap water to cultivate *Chlorophycae*, *Chlorococcum* and *Cyanophycee*.

References: Beijerinck (1893 p. 371), Beijerinck (1901 p. 563).

112. Nabokoch and Lebedeff's Hydrogen Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. Na_2HPO_4	0.5 g.
3. KNO_3	2.0 g.
4. $MgSO_4$	0.2 g.
5. $NaHCO_3$	1.0 g.
6. Fe_2Cl_6	some
7. Hydrogen	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) The reaction is slightly alkaline (about 0.050 g. H_2SO_4 to 100.0 cc. of solution).

(3) Distribute in 100 or 150.0 cc. lots in vacuum flasks with side tubes.

(4) Inoculate.

(5) Remove all the air by means of an oil pump.

(6) Replace the air by a mixture of oxygen and hydrogen ("Knallgase") containing carbonic acid.

Sterilization: Method not given.

Use: To study the oxidation of hydrogen by microörganisms.

Reference: Nabokich and Lebedeff (1907 p. 352), Löhnis (1913 p. 106).

113. Bokorny's Calcium Nitrate Solution

Constituents:

1. Water	100.0 cc.
2. $Ca(NO_3)_2$	0.1 g.
3. $MgSO_4$ (crystalline)	0.02 g.
4. KH_2PO_4	0.02 g.
5. Iron chloride	trace
6. KCl	0.0 or 0.05 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study the metals required for the growth of *Spirogyra majuscula*. It was found that potassium was necessary.

Reference: Bokorny (1912 p. 125).

SUBGROUP I-C

Liquid Media with One or More Constituents Organic

The liquid media in which one or more constituents are organic may be divided conveniently into sections based upon the character of the nitrogen and carbon sources supplied. The sections recognized may be differentiated as follows:

Key to the sections of Subgroup I-C

A_1. Chemical composition of all constituents known.

B_1. Nitrogen inorganic, carbon organic.

C_1. Nitrogen present as free or elementary nitrogen only...Section 1 (Med. 114–190)

C_2. Nitrogen present as ammonium salts.
Section 2 (Med. 191–323)

C_3. Nitrogen present as nitrites.
Section 3 (Med. 324–327)

C_4. Nitrogen present as nitrates.
Section 4 (Med. 328–365)

B_2. Nitrogen organic.

C_1. Nitrogen present as amino acids.
Section 5 (Med. 366–479)

C_2. Organic nitrogen other than amino acids present...Section 6 (Med. 480–515)

A_2. Chemical composition of one or more constituents not definitely known.

B_1.* Containing digests.

C_1.* Containing commercial peptones or digests.

D_1. Additional constituents, if any, inorganic.........Section 7 (Med. 516–555)

D_2. Containing additional organic constituents.

E_1. Chemical composition of all additional materials known.

F_1. No additional nitrogen compounds added.

G_1. Basal solutions. Employed with the addition of various nutrients.
Section 8 (Med. 556–588)

B_2. Complete nutrient solutions.
Section 9 (Med. 589–647)

F_2. At least one additional nitrogenous constituent.
Section 10 (Med. 648–683)

E_2. Composition of one or more additional materials not definitely known.

F_1. Additional organic material exclusively of plant origin
Section 11 (Med. 684–712)

F_2. Containing additional materials of animal origin; plant derivatives may also be present.

G_1. Not containing extracts or infusion.
Section 12 (Med. 713–747)

G_2. Containing extracts or infusion.
Section 13 (Med. 748–977)

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* See page 37 for B_2 and C_2.

C_2. Containing non-commercial digests.
Section 14 (Med. 978–1140)
B_2. Not containing digests.
C_1. Containing plant constituents of unknown composition.
Section 15 (Med. 1141–1228)
C_2. Containing animal constituents of unknown composition.
Section 16 (Med. 1229–1376)
C_3. Containing extracts of soil, ashes, etc.
Section 17 (Med. 1377–1394)

SUBGROUP I-C. SECTION 1

Liquid media or basal solutions with constituents of known chemical composition containing free or elementary nitrogen only, carbon organic.

A_1. Inorganic salts not added.
B_1. Carbohydrates added.
Pringshein's Glucose Cellulose Solution 114
Waksman and Joffe's Basal Glucose Solution 115
Buchanan's Sucrose Solution 116
B_2. Alcohols added.
Waksman and Joffe's Basal Glycerol Solution 117
Buchanan's Glycerol Solution I 118
A_2. Inorganic salts added.
B_1. Potassium salts, only, added.
C_1. Containing carbohydrates.
Beijerinck and van Delden's Phosphate Glucose solution 119
Beijerinck's Phosphate Sucrose Solution 120
C_2. Containing alcohols.
Beijerinck's Phosphate Mannitol Solution 121
C_3. Containing salts of organic acids.
van Delden's Basal Lactate Solution. 122
Beijerinck and van Delden's Acetate Solution 123
B_2. Other salts added.
C_1.* Magnesium salts added.
D_1. Phosphates not added.
Gage's Glucose Salt Solution 124
Stoklasa's Phosphorous Free Glucose Salt Solution 125
D_2. Phosphates added.
E_1.* Calcium salts not added.
F_1. Carbon present as carbohydrates.
G_1. Monosaccharides added.
Gerlach and Vogel's Basal Glucose Salt Solution 126
Henneberg's Basal Glucose Di-sodium Phosphate Solution 127
Beijerinck's Glucose Salt Solution ... 128
Kisch's Basal Glucose Salt Solution. 129
Beijerinck's Phosphate Glucose Salt Solution (Harvey No. 1) 130
Laborde's Basal Invert Sugar Tartarate Solution 131
Calmette, Massol and Breton's Basal Glucose Salt Solution 132
Löhnis' Basal Glucose Salt Solution .. 133
G_2. Disaccharides added.
Will's Basal Sucrose Salt Solution ... 134
Henneberg's Basal Sucrose Salt Solution 135
Czapek's Sucrose Salt Solution (Waksman) 136
von Bronsart's Basal Sucrose Salt Solution 137
Bokorny's Basal Sucrose Salt Solution 138
Smith's Sucrose Salt Solution 139
Munter's Basal Galactose Salt Solution 140
Buchanan's Basal Maltose Salt Solution 141
G_3. Polysaccharides Added.
Omeliansky's Basal Cellulose Salt Solution 142
F_2.* Carbon present as alcohols.
G_1. Glycerol added.
Percival's Glycerol Phosphate Solution 143
Buchanan's Glycerol Salt Solution II. 144
Gerlach and Vogel's Basal Glycerol Salt Solution 145
Kuhne's Basal Lactate Solution. (Proskauer and Beck) 146
Calimard and Lacomme's Basal Glycerol Salt Solution (Kolle-Wasserman) 147
G_2. Mannitol added.
Bijerinck's Modified Mannitol Salt Solution. (Omeliansky and Sswerowa) 148
Murray's Mannitol Salt Solution ... 149
Percival's Mannitol Phosphate Solution 150
Capaldi and Proskauer's Basal Mannitol Salt Solution 151

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* See next page for C_2, E_2.

* See next page for F_3.

F_3. Carbon present as organic acids or their salts.
Percival's Succinate Solution....... 152
E_2. Calcium salts added.
F_1. Carbon present as carbohydrates.
G_1. Glucose added.
H_1. Glucose only organic carbon source.
Winogradsky's Salt Glucose Solution. 153
Stutzer, Burri and Maul's Glucose Salt Solution...................... 154
Stoklasa's Basal Glucose Salt Solution.............................. 155
Charpentier's Salt Glucose Solution. 156
Krzemieniewska's Glucose Salt Solution (Vogel)...................... 157
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Charpentier's Basal Glucose Salt Solution......................... 159
Stoklasa's Basal Glucose Salt Solution.............................. 160
Henneberg's Basal Glucose Salt Solution.............................. 161
H_2. Glucose with other organic carbon.
Gottheil's Carbohydrate Glycerol Solution......................... 162
Harvey's Citric Acid Glucose Solution.............................. 163
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G_2. Sucrose added.
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Mazé's Sucrose Salt Solution........ 166
Linde's Basal Sucrose Salt Solution.. 167
G_3. Polysaccharides added.
Löhnis and Lochhead's Basal Cellulose Salt Solution................. 168
van Iterson's Basal Cellulose Salt Solution......................... 169
F_2.* Carbon present as alcohols.
G_1. Mannitol added.
Jones' Modified Ashby's Mannitol Solution 170
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Harvey's Mannitol Salt Solution.... 173
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G_2. Glycerol added.
Koser and Rettger's Basal Glycerol Salt Solution..................... 176
F_3. Carbon present as organic acids or their salts.
Puriewitsch's Tartaric Acid Salt Solution......................... 177
C_2. Magnesium salts not added.
D_1. Phosphates not added.
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D_2. Phosphates added.
E_1. Calcium salts not added.
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E_2. Calcium salts added.
F_1. Carbon present as carbohydrates.
Bijerinck's Phosphate Glucose Solution.............................. 185
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F_2. Carbon present as alcohols or acids.
Wojtkiewicz's Mannitol Salt Solution.............................. 187
Kranisky's Mannitol Salt Solution... 188
Peklo's Modified Bijerinck's Mannitol Salt Solution................ 189
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114. Pringsheim's Glucose Cellulose Solution

Constituents:

1. Water................	1000.0 cc.
2. Cellulose............	25.0 g.
3. Glucose..............	0.2 or 0.4 g.

Preparation: (1) Add 2 and 3 to 1.

Sterilization: Not specified.

Use: To study nitrogen assimilation methane fermenting bacteria.

Variants: The author used the following combinations to study nitrogen assimilation by clostridium and azotobacter:
(a) 10.0 g. cellulose +0.4 g. dextrose in 500.0 cc. solution
(b) 5.0 g. cellulose +0.4 g. dextrose in 250.0 cc. solution
(c) 10.0 g. cellulose +0.2 g. dextrose in 1000.0 cc. solution

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* See next column for F_3.

(d) 5.0 g. cellulose +0.2 g. dextrose in 1000.0 cc. solution
(e) 2.5 g. cellulose +0.2 g. dextrose in 500.0 cc. solution
(f) 5.0 g. cellulose +0.2 g. dextrose in 500.0 cc. solution
(g) 2.5 g. cellulose +0.2 g. dextrose in 500.0 cc. solution
(h) 5.0 g. cellulose + 0.2 g. mannite in 1000.0 cc. solution

Reference: Pringsheim (1909 p. 303), (1910 p. 224).

115. Waksman and Joffe's Basal Glucose Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose	30.0 g.

Preparation:
(1) Dissolve 2 in 1.
(2) Add 2.0 g. of one of the added nutrients.
(3) Tube in 10 to 12 cc. lots.

Sterilization: Sterilize at 15 pounds for 15 minutes.

Use: To study reaction changes in Actinomycetes metabolism.

Added nutrients: The authors added 2.0 g. of one of the following nitrogen sources:

$(NH_4)_2SO_4$	(pH of solution = 5.8)
$(NH_4)_2CO_3$	(pH of solution = 6.8)
Urea	(pH of solution = 7.4)

Reference: Waksman and Joffe (1920 p. 39).

116. Buchanan's Sucrose Solution

Constituents:

1. Water (tap)	1000.0 cc.
2. Sucrose (2.0%)	20.0 g.

Preparation:
(1) Prepare a 2.0% saccharose solution in tap water.
(2) Distribute in 500.0 cc. lots in liter flasks.

Sterilization: Method not given.

Use: Development of gum produced by *Bacillus radicicola*. The author found that 1.0% N/1 malic acid or citric acid was sufficient to completely inhibit growth. 1.0% N/1 NaOH stimulated growth and gum production, while 2.0% prevented growth. To isolate the gum add 100.0 cc. of 95% alcohol, to the 2 week old culture, filtering the white precipitate, wash in alcohol and dissolve in distilled water. Repeat the precipitation 5 times. Finally dry the precipitate. Saccharose may be used in higher concentrations.

Reference: Buchanan (1909 p. 371).

117. Waksman and Joffe's Basal Glycerol Solution

Constituents:

1. Water to make	1000.0 cc.
2. Glycerol	30.0 g.

Preparation:
(1) Dissolve 2 in 1.
(2) Add one of the test materials (added nutrients) to (1). When using casein and egg albumin, dissolve casein and egg albumin in N/10 NaOH. In using fibrin, add fibrin to each tube.
(3) Solutions vary in pH = 7.2 to 7.7, urea pH = 8.0.
(4) Tube in 10–12 cc. lots.

Sterilization: Autoclave for 15 minutes at 15 pounds pressure.

Use: To study change in reaction in *Actinomycetes* metabolism.

Added nutrients: The authors added 5.0 g. of one of the following nitrogenous compounds:

fibrin
casein
egg albumin
peptone
asparagin
leucin
glycocoll
urea
acetamide 5.0 g.

Variants: The authors used 3.0 g. of glycerol and added 2.0 g. of $NaNO_3$, $NaNO_2$, or $(NH_4)_2SO_4$ as inorganic nitrogen sources.

Reference: Waksman and Joffe (1920 pp. 36–37).

118. Buchanan's Glycerol Solution I

Constituents:

1. Water (tap)	1000.0 cc.
2. Glycerol	5.0 cc.

Preparation: (1) Dissolve 2 in 1.

Sterilization: Not specified.

Use: Cultivation of *Monascus purpurens* from silage.

Variants: The author used a 10.0, 20.0 or 40.0% glycerol solution instead of 5.0%. Growth in the 5.0 and 10.0% solution, but little or no growth in the 20.0 or 40.0% solutions.

Reference: Buchanan (1910 p. 102).

119. Beijerinck and van Delden's Phosphate Glucose Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose	20.0 g.
3. K_2HPO_4	0.5 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not given.

Use: To study nitrogen assimilation by chroococcum.

Variants: Stoklasa prepared a solution of 25.0 g. of glucose and 1.5 g. K_2HPO_4 in 1000.0 cc. of Moldau river water. He studied the relation between the assimilation of phosphoric anhydride and nitrogen by azotobacter. It was determined that 1.0 g. of phosphoric acid anhydride and 2 to 2.3 g. elementary N were assimilated.

Reference: Beijerinck and van Delden (1902 p. 8), Stoklasa (1911 p. 461).

120. Beijerinck's Phosphate Sucrose Solution

Constituents:

1. Water	1000.0 cc.
2. Sucrose	20.0 g.
3. K_2HPO_4	0.2 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: Growth of *Bacillus radicicola*.

Reference: Beijerinck (1901 p. 575).

121. Beijerinck's Phosphate Mannitol Solution

Constituents:

1. Water	1000.0 cc.
2. Mannitol	20.0 g.
3. K_2HPO_4	0.2 g.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Reaction is slightly alkaline.
(3) Inoculate with 0.1 to 0.2 g. earth.

Sterilization: Not specified.

Use: Used for the enrichment of *Azotobacter chroococcum* and for nitrogen assimilation studies.

Variants:

(a) Beijerinck specified the use of ditch water.
(b) Jacobitz specified the use of distilled water and studied nitrogen fixation by *Bacillus ellenbachensis* α (Caron).
(c) Christensen specified distilled water and suggested that $CaCO_3$ might be added if desired.
(d) Percival specified the use of tap water.
(e) Beijerinck and van Delden used 0.5 g. K_2HPO_4 instead of 0.2 g. K_2HPO_4.
(f) Stoklasa specified Moldau river water and used 0.5 g. potassium phosphate instead of 0.2 g. K_2HPO_4.

Reference: Beijerinck (1901 pp. 568, 578), Jacobitz (1903 p. 101), Christensen (1907 p. 110), Percival (1920 p. 179), Beijerinck and Van Delden (1902 p. 8), Stoklasa (1908 p. 489).

122. van Delden's Basal Lactate Solution

Constituents:

1. Water (ditch)	1000.0 cc.
2. K_2HPO_4	0.2 g.
3. Sodium lactate	1.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Additional nutrients may be added as indicated below.

Sterilization: Not specified.

Use: Cultivation and enrichment of *Microspira desulfuricans*. Used also to study sulphate reduction.

Variants:

(a) The author used 0.5 g. sodium lactate.
(b) The author used 0.5 g. K_2HPO_4 and 1.0 g. of calcium lactate.

Added nutrients:

(a) The author did not specify the use of ditch water, and added $(NH_4)_2SO_4$ and 0.6 g. $MgSO_4 \cdot 7H_2O$.
(b) The author did not specify the use of ditch water and added 0.2 g. glycocoll and 0.6 g. $MgSO_4 \cdot 7H_2O$.
(c) The author used 0.5 g. sodium lactate with 0.5 g. K_2HPO_4 and added 1.0 g. asparagin and 0.0, 0.3, 0.6, 0.9, or 1.2 g. gypsum.
(d) The author specified the use of tap water, used 0.5 g. sodium lactate with 0.5 K_2HPO_4, added NH_4Cl and 1.2 g. gypsum.
(e) The author specified the use of tap water, used 0.5 g. of sodium lactate with 0.5 g. K_2HPO_4 and added 0.5 g. asparagin.
(f) The author specified the use of tap water, used 0.5 g. of sodium lactate with 0.5 g. K_2HPO_4 added 1.0 g.

asparagin and 1.0 g. gypsum or 4.0 g. $MgSO_4\cdot 7H_2O$.

(g) The author specified the use of tap water, used 0.5 g. of sodium lactate with 0.5 g. K_2HPO_4 added 2.5 g. asparagin and 4.0 g. $MgSO_4\cdot 7H_2O$.

(h) The author specified the use of tap water, used 10.0 g. sodium lactate, added 1.0 g. of asparagin and 2.0 or 5.0 g. of $MgSO_4\cdot 7H_2O$.

Reference: van Delden (1903–04 p. 85).

123. Beijerinck and van Delden's Acetate Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	0.5 g.
3. Sodium acetate	5.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: Cultivation of chroococcum and granulobacter. The medium was inoculated with 20.0 g. of fresh earth.

Reference: Beijerinck and van Delden (1902 p. 29).

124. Gage's Glucose Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose	5.0 g.
3. $MgSO_4$	0.25 g.
4. $CaCl_2$	0.01 g.
5. Iron sulphate solution	1 drop

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: To study the fixation of nitrogen by *Pseudomonas radicicola* and nitroso bacteria. It was found that nitrites and possibly nitrates were produced by *Bacillus radicicola* in this medium.

Reference: Gage (1910 p. 34).

125. Stoklasa's Phosphorus Free Glucose Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. d-glucose	25.0 g.
3. K_2SO_4	1.0 g.
4. $MgCl_2$	0.5 g.
5. Iron sulphate	0.1 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: To study the rôle of phosphorus in the soil. It was found that growth of nitrogen fixing organisms was very poor in this solution. Phosphorus is required for luxuriant and rapid development.

Variants:

(a) The author suggested the addition of 1.0 g. of nitrogen in the form of $(NH_4)_2SO_4$ or $NaNO_3$.

(b) The author suggested the addition of 0.1 g. of aluminum sulphate and 1.0 g. of one of the following phosphorus compounds:

dicalcium phosphate $CaHPO_4\cdot 2H_2O$.
Monodiferric phosphate $Fe_2O_3(P_2O_5)_3$.
Monodialuminum phosphate $Al_2O_3(P_2O_5)_2\cdot 8H_2O$.
Tricalcium phosphate $Ca_3(PO_4)_2\cdot 2H_2O$.
Ditriferric phosphate $(FeO_3)_4(P_2O_5)_5\cdot 3H_2O$.
Triferric phosphate $Fe_2O_3P_2O_5 + 4H_2O$.
Trialuminum phosphate $Al_2O_3\cdot P_2O_5 + 4H_2O$.

(c) The author used variant (b) and substituted arabniose for d-glucose.

(d) The author added aluminum sulphate and the equivalent of 0.2 g. of phosphoric anhydride in the form of monomagnesium phosphate, monodialuminum phosphate, monodiferric phosphate or trimagnesium phosphate to the original solution.

References: Stoklasa (1911 pp. 492, 441, 490).

126. Gerlach and Vogel's Basal Glucose Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Glucose	5.0 g.
3. Potassium phosphate	0.5 g.
4. $MgSO_4$	0.3 g.
5. NaCl	0.5 g.
6. Na_2CO_3	0.5 g.
7. $FeSO_4$	0.2 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Add one of the nitrogen sources listed in added nutrients.
(3) Adjustment of reaction not given.

Sterilization: Method not specified. When utilizing urea as nitrogen source sterilize the urea at 100°C. dry heat.

Use: Cultivation of albumin formers from soil and stable manure. These organisms grow very well on agar, gelatin and bouillon which contain 0.3% $NaNO_3$. The nitrate in this medium is changed to nitrite after a few days and then quantitively to insoluble albuminous materials. Ammonia salts and urea also are suitable nitrogen sources for albumin production by these organisms. Albumin production using urea however goes on very slowly.

Added nutrients: The author employed the following nitrogen sources:

$NaNO_3$ (c.p. Merck)	3.0 g.
$(NH_4)_2SO_4$	3.0 g.
NH_4NO_3	2.0 g.
NH_4CO_3	2.0 g.
urea	20.0 g.

Reference: Gerlach and Vogel (1901 p. 612).

127. Henneberg's Basal Glucose Disodium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. Na_2HPO_4	3.0 g.
3. $MgSO_4$	2.0 g.
4. NaCl	2.0 g.
5. Glucose	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add one of the added nutrients (amounts not specified).

(3) Adjustment of reaction not given.

Sterilization: Method not given.

Use: To study the food requirements for acetic acid organisms, *B. Pasteurianum*, *B. oxydans*, *B. aceti* and *B. Kützingianum*. The author found that KNO_3 was the poorest source of nitrogen and $(NH_4)_2SO_4$ was the best.

Added nutrients: The author employed one of the following salts or carbon sources:

KNO_3
$(NH_4)_2SO_4$
ammonium tartrate
asparagin
peptone

Reference: Henneberg (1898 p. 18).

128. Beijerinck's Glucose Salt Solution

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4	0.5 g.
3. $MgSO_4$	0.5 g.
4. Glucose	10.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Growth of *Streptothrix chromogena*.

Variants:

(a) Peklo used the solution to grow plant actinomyces. He specified that the solution might be neutralized by the addition of 0.5 g. Na_2CO_3.

(b) Malenkovic used 100.0 g. glucose, 1.0 g. K_2HPO_4 and added 2.0 g. of KNO_3 or 2.0 g. $NH_4H_2PO_4$. He cultivated coniophora cerebello (*Corticium putaneum*) and reported $NH_4H_2PO_4$ a better nitrogen source.

Reference: Beijerinck (1900 p. 7), Peklo (1910 p. 514). Malenkovic (1906 p. 412).

129. Kisch's Basal Glucose Salt Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $MgSO_4$	0.5 g.
4. NaCl	0.02 g.
5. $FeSO_4$	trace
6. Calcium phosphate	trace
7. Glucose	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Add Na_2CO_3 until the reaction is slightly alkaline to litmus.

(3) Add one of the nitrogen sources listed under added nutrients to (2).

Sterilization: Not specified.

Use: To study the nitrogen requirements of members of the colon-typhoid group.

Added nutrients: The author added one of the following nitrogen sources.

(a) KNO_3 .... 0.29 g.
Bact. paratyphi B. shows growth. *Bact. enteritidis* Gärtner little growth.

(b) KNO_2 .... 2.43 g.
No growth.

(c) Urea .... 8.6 g.
Bact. coli and *Bact. enteritidis* Gärtner show some growth.

(d) Urea .... 8.6 g.
KNO_2 .... 2.4 g.
B. coli shows growth.

(e) $(NH_4)_2SO_4$ .... 1.9 g.
B. paratyphi shows good growth. *B. coli* shows good growth. *B. enteritidis Gärtner*, and dysentery bacillus show good growth.

(f) $NH_4H_2PO_4$ .... 1.42 g.
Bact. paratyphi shows good growth.

Bact. coli shows good growth. *B. enteritidis* Gärtner and dysentery bacillus show good growth.

(g) NH_4Cl .... 1.63 g. *Bact. coli* and *B. enteritidis* show slight growth. Also *B. paratyphi*.

(h) $(NH_4)_2CO_3$ .... 1.1 g. *Bact. coli* and *B. enteritidis* show slight growth. Also *B. paratyphi*.

(i) Ammonium lactate .... 2.94 g. *B. typhi*, *B. paratyphi*, *B. enteritidis*, Gärtner, *B. typhi* murum and dysentery show slight growth. *Bact. coli* grows well.

(j) Ammonium tartrate .... 2.62 g. *B. paratyphi*, *Bact. coli*, *B. enteritidis* Gärtner show good growth. *B. dys.* Flexner show growth.

(k) Asparagin .... 1.9 g. Nearly all strains show growth.

(l) Nucleic acid (from yeast Merck .... 2.6 g. Nearly all except *B. paratyphi A* show growth.

Reference: Kisch (1918–19 p. 32).

130. Beijerinck's Phosphate Glucose Salt Solution (Harvey No. I)

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $MgSO_4$	0.2 g.
4. NaCl	0.02 g.
5. 1–1000 $MnSO_4$	1.0 cc.
6. 1–1000 $FeSO_4$	1.0 cc.
7. Glucose	20.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Study of nitrogen fixation.

Variants:

(a) Giltner used 0.5 g. $MgSO_4$, 0.01 g. $Fe_2(SO_4)_3$, 0.01 g. $MnSO_4$ and 0.01 g. NaCl.

(b) Heinemann used 0.5 g. $MgSO_4$, 0.01 g. $FeSO_4$, 0.01 g. $MnSO_4$ and 0.01 g. NaCl.

Reference: Harvey (1921–22 p. 105), Giltner (1921 p. 370), Heinemann (1922 p. 39).

131. Laborde's Basal Invert Sugar Tartrate Solution

Constituents:

1. Water	1000.0 cc.
2. Invert sugar	50.0 g.
3. Tartaric acid	2.5 g.
4. Potassium tartrate (neutral)	0.75 g.
5. $Mg_3(PO_4)_2$	1.0 g.
6. H_2SO_4	0.1 g.
7. Iron and zinc sulphate	trace
8. K_2SiO_3	trace

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: To study the nitrogen requirements of a mold, *Eurotiopsis Gayoni*.

Added nutrients: The author added 0.2 g. of nitrogen in the form of one of the following:

$NaNO_3$
KNO_3
Ammonium tartrate
NH_4Cl
$(NH_4)_2SO_4$
Ammonium phosphate
NH_4NO_2
gluten
casein
asparagin
urea
gelatin
fibrin
blood albumin
egg albumin
yeast extract
peptone

Reference: Laborde (1897 p. 3).

132. Calmette, Massol and Breton's Basal Glucose Solution

Constituents:

1. Water	1000.0 cc.
2. $FeSO_4$	0.04 g.
3. $MgSO_4$	0.05 g.
4. K_2HPO_4	1.05 g.
5. NaCl	8.5 g.
6. Glucose	10.0 g.
7. Na_2CO_3	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Add one of the added nutrients to (1).

Sterilization: Not specified.

Use: To determine constituents essential for growth of tubercle bacilli. The authors reported that generally inorganic nitrogen did not support the growth of the tubercle bacillus.

Added nutrients: The authors prepared the following solutions.

(a) Basal solution + 10.0 g. Na_2CO_3 + 5.0 g. of one of the nitrogen sources.

(b) Basal solution + 10.0 g. Na_2CO_3 + 40.0 g. of glycerol + 5.0 g. of one of the nitrogen sources.

(c) Basal solution + 40.0 g. glycerol + 5.0 g. of one of the nitrogen sources.

The following nitrogen sources were given:

$NaNO_3$
$NaNO_2$
$(NH_4)_2SO_4$
$(NH_4)_2HPO_4$
Ammonium acetate
Ammonium tartrate
Ammonium citrate
Ammonium malate
Ammonium succinate
Succinamide
Succinimide
Asparagin
Leucin
Tyrosine

Reference: Calmette, Massol and Breton (1909 p. 581).

133. Löhnis' Basal Glucose Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	0.5 g.
3. $MgSO_4$	0.02 g.
4. NaCl	0.02 g.
5. Fe_2Cl_6	trace
6. Glucose (1.0%)	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Dissolve 1.0% glucose and 0.1% of one of the added nutrients in (1).

Sterilization: Not specified.

Use: To study nitrogen assimilation.

Added nutrients: The author added 0.1% of one of the following:

Urea
sodium hippurate
asparagin
ammonium lactate
uric acid
$(NH_4)_2SO_4$
ammonium acetate
ammonium butyrate
$NaNO_3$

Reference: Löhnis (1913 p. 99).

134. Will's Basal Sucrose Solution

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4	25.0 g.
3. $MgSO_4$	8.5 g.
4. Sucrose	5.0 g.
5. Citric acid	5.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Add 0.5% citric acid to (1).
(3) Add one of the combinations given under added nutrients.

Sterilization: Method not given.

Use: Cultivation of yeast.

Added nutrients: The author added one of the following combinations to the basic solution:

(a) asparagin	20.0 g.
dextrin	30.0 g.
(b) peptone	20.0 g.
dextrin	30.0 g.

Variants: Peklo used a solution containing 5.0 g. KH_2PO_4, 2.0 g. $MgSO_4$ and 20.0 g. of sucrose per liter to grow plant actinomyces.

Reference: Will (1896 p. 754), Peklo (1910 p. 551).

135. Henneberg's Basal Sucrose Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4 (0.5%)	5.0 g.
3. $MgSO_4$ (0.2%)	2.0 g.
4. Sucrose	50.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Add one of the added nutrients.

Sterilization: Not specified.

Use: Cultivation of yeast.

Added nutrients: The author added 0.3% of one of the following as nitrogen sources:

ammonium tartrate
$(NH_4)_2SO_4$; NH_4Cl
ammonium phosphate, NH_4NO_3

Reference: Henneberg (1907 #40–50) (1909 p. 104).

136. Czapek's Sucrose Salt Solution (Waksman)

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. KCl	0.5 g.
4. $MgSO_4$	0.5 g.
5. $FeSO_4$	0.01 g.
6. Sucrose	30.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Place in 100.0 cc. portions in flasks.

Sterilization: Autoclave at 15 pounds pressure for 15 minutes.

Use: Cultivation of bacteria found in soil.

Reference: Waksman (1918 p. 479).

137. von Bronsart's Basal Sucrose Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Sucrose (4.0%)	40.0 g.
3. $MgSO_4$ (0.25%)	2.5 g.
4. KH_2PO_4 (0.25%)	2.5 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add one of the nitrogen sources given in the added nutrients.

Sterilization: Not specified.

Use: To study the availability of various nitrogen sources for *Xylaria*, *Xylaria arbuscula*, *Xylaria polymorpha*, *Xylaria hypoxylon*. The author found that asparagin was a better nitrogen source than peptone. KNO_3 was found to be the best inorganic source.

Variants: The author used 0.5% asparagin with 1.0, 4.0, 8.0, 16.0, 32.0 or 50.0% sucrose. He found that sugar concentrations above 16.0% inhibited development of the Xylaria listed above. There was slight growth using 32.0% sucrose, but no growth using 50.0% sucrose.

Added nutrients: The author employed the following nitrogen sources:

asparagin	1.0%
peptone	0.5%
NH_4NO_3	1.0%
$(NH_4)_2SO_4$	0.5%

Reference: von Bronsart (1919 p. 61).

138. Bokorny's Basal Sucrose Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Sucrose	100.0 g.
3. $MgSO_4$	1.0 g.
4. KH_2PO_4	5.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add one of the materials listed under added nutrients.

Sterilization: Not specified.

Use: To study yeast growth.

Added nutrients and variants: One of the following nitrogen sources or combinations was added:

(a) asparagin	25.0 g.
(b) $(NH_4)_2SO_4$	5.0 g.

(c) asparagin 5.0 g. with 1.0 g. KH_2PO_4, a trace of $CaCl_2$ and varying amounts of $MgSO_4$, 0.3 g. to 20.0 g. for the basic solution. The author reported that 2.0% $MgSO_4$ did not inhibit the growth of yeast, but 0.03% sufficed for normal growth.

(d) peptone	25.0 g.

(e) aspartic acid—amount not given.

(f) leucine—amount not given.

(g) tyrosine—amount not given.

(h) glycocoll—amount not given.

(i) Bokorny used 4.0 g. KH_2PO_4 in the basic solution and added one of the following:

asparagin	25.0 g.
aspartic acid	25.0 g.
peptone	25.0 g.

(j) Bokorny used 4.0% sucrose in the basic solution, and added 0.25% aspartic acid.

(k) Bokorny used 1.0 g. KH_2PO_4, 0.3 g. $MgSO_4$ and added a trace of $CaCl_2$ to the basic solution. To this solution he added:

(a) KNO_3, 0.1, 0.2 or 0.5 g.

(b) KNO_3, 0.2 and asparagin 2.0 g.

(c) KNO_3, 0.1 g. and asparagin 0.3 g.

Reference: Bokorny (1911 p. 183; 1912 pp. 121; 148; 1917 p. 364; 1917 p. 47; 1920 p. 24).

139. Smith's Sucrose Salt Solution

Constituents:

1. Triple distilled water	1000.0 cc.
2. Sucrose	5.0 cc.
3. KH_2PO_4	2.0 cc.
4. $MgSO_4 \cdot 7H_2O$	0.1 cc.
5. NaCl	0.5 cc.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) All apparatus should be scrupulously clean and only chemicals of known purity be used.

Sterilization: Not specified.

Use: Cultivation of bacteria.

Reference: Smith (1905 p. 198), Tanner (1919 p. 65).

140. Münter's Basal Galactose Salt Solution

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4$	0.5 g.
3. NaCl	0.5 g.
4. K_2HPO_4	1.5 g.
5. $CaCl_2$	0.1 g.
6. $FeCl_3$	drops

7. Glucose	10.0 g.
8. Galactose	2.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.
(2) Distribute in 50.0 cc. lots.
(3) Add 0.25 g. of one of the added nutrients to each 50.0 cc. lot.

Sterilization: Not specified.

Use: Cultivation of Actinomycetes. *Actinomyces odorifer*, *Act. chromogenes* and others. Alanin and tyrosin were found to be the best nitrogen sources for the actinomycetes studied. The remainder of the compounds, however, were good sources for some of the actinomycetes.

Added nutrients: The author added 0.25 g. of one of the following to each 50.0 cc. of solution:

urea
sulphocarbamide
alanin
tyrosin
dicyandiamide

Reference: Münter (1913 p. 377).

141. Buchanan's Basal Maltose Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4	2.0 g.
3. $MgSO_4$	0.1 g.
4. Maltose (1.0%)	10.0 g.

Preparation:

(1) Add 2, 3, and 4 to 1.
(2) Add 1.0% of one of the added nutrients.

Sterilization: Sterilize for 20 minutes on each of 3 successive days in streaming steam. Then incubate at room temperature for one week to test sterility.

Use: To study gum formation by *Bacillus radicicola*. Growth and gum production generally good. Poorest results were obtained when ammonium citrate was present in the medium.

Added nutrients: The author added 1.0% of one of the following:

Ammonium succinate
Ammonium citrate
Ammonium asparaginate
Asparagin
Peptone (Witte's)

Reference: Buchanan (1909 p. 393).

142. Omeliansky's Basal Cellulose Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. NaCl	trace
3. $MgSO_4$	0.5 g.
4. Potassium phosphate	1.0 g.
5. Filter paper, straw, lilac pith, or cabbage	

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Add one of the added nutrients.
(3) Add one of the cellulose sources.

Sterilization: Not specified.

Use: To study fermentation of cellulose by amylobacter and organisms found in the mud of rivers.

Added nutrients and variants:

(a) The author added 1.0 g. of one of the following to the basic solution:

$(NH_4)_2SO_4$
ammonium phosphate
peptone
asparagin

(b) Kroulik specified the use of 1.0 g. K_2HPO_4 in the basic solution and added 1.0 to 2.0% cellulose and added 1.0 g. $(NH_4)_2SO_4$, 0.5% $CaCO_3$, and 1.0% $MgCO_3$. He used the medium for the cultivation of cellulose splitting thermophiles.

(c) Brussoff specified the use of K_2HPO_4 in the basic solution and added 1.0 g. $(NH_4)_2SO_4$, 2.0 g. $CaCO_3$ and filter paper. The solution was distributed in 20.0 cc. lots and a strip of filter paper (about 0.1 g.) was added to each lot. The medium was used to study cellulose decomposition by iron bacteria (*Ferribacterium duplex*). He reported that gas bubbles were formed after 2 days and a yellow membrane formed after 4 days when inoculated with sludge.

(d) Khouvine specified the use of K_2HPO_4 in the basic solution and added 1.0 g. $(NH_4)_2SO_4$ 0.0 or 2.0 g. of glucose and cellulose. The medium was tubed in 5.0 cc. lots and 1 square centimeter of Berzilius filter paper, or a little cellulose precipitated after having been dissolved in Schweitzer's reagent to each tube.

The medium was used for isolation and enrichment of *B. cellulose dissolvens*.

(e) Giltner specified the use of K_2HPO_4 in the basic solution and added 1.0 g. $(NH_4)_2SO_4$ and added 2.0 g. of filter paper, cotton straw or starch. The medium was used to cultivate anaerobic cellulose fermenters.

Reference: Omeliansky (1902 p. 226), Kroulik (1912–13 p. 340), Brussoff (1918 p. 193), Giltner (1921 p. 373), Khouvine (1923 p. 713).

143. Percival's Glycerol Phosphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Magnesium phosphate	0.1 g.
3. KH_2PO_4	2.0 g.
4. Glycerol	10.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Add 4 to (1).
(3) Heat to boiling.
(4) Filter.
(5) Adjustment of reaction not specified.
(6) Tube in 10 to 15.0 cc. quantities.
(7) After sterilization inoculate with contents of a nodule of a pea, runner bean, red clover and broad bean by means of a sterile knife.

Sterilization: Steam on each of 3 successive days.

Use: Growth of bacteria from the roots of leguminous plants, (Bacteroids).

Reference: Percival (1920 p. 204).

144. Buchanan's Glycerol Salt Solution II

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4 (0.2%)	2.0 g.
3. $MgSO_4$ (0.01%)	0.1 g.
4. Glycerol (1.0, 3.0 or 5.0%)	10.0, 30.0 or 50.0 g.

Preparation:

(1) Heat 2, 3 and 4 in 1 to boiling.
(2) Cool on ice.
(3) Filter to remove insoluble precipitates.
(4) Tube.

Sterilization: Sterilize intermittently in flowing steam on each of 3 successive days.

Use: Cultivation of *Bacillus radicicola* bacteroids.

Variants: The author added (0.5%) 5.0 g. ammonium phosphate with (1.0%) 10.0 g. glycerol.

Reference: Buchanan (1909 p. 62).

145. Gerlach and Vogel's Basal Glycerol Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Glycerol	5.0 g.
3. Potassium phosphate	0.5 g.
4. $MgSO_4$	0.3 g.
5. NaCl	0.5 g.
6. Na_2CO_4	0.5 g.
7. $FeSO_4$	0.2 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Add one of the nitrogen sources listed in added nutrients.
(3) Adjustment of reaction not given.

Sterilization: Method not specified. When utilizing urea as nitrogen source sterilize the urea at 100°C. dry heat.

Use: Cultivation of albumin formers from soil and stable manure. These organisms grow very well on agar, gelatin and bullion which contain 0.3% $NaNO_3$. The nitrate in this medium is changed to nitrite after a few days and then quantitatively to insoluble albuminous materials. Ammonia salts and urea also are suitable nitrogen sources for albumin production by these organisms. Albumin production using urea, however, goes on very slowly.

Added nutrients and variants:

(a) The author employed the following nitrogen sources:

$NaNO_3$ (c.p. Merck)	3.0 g.
$(NH_4)_2SO_4$	3.0 g.
NH_4NO_3	2.0 g.
NH_4CO_3	2.0 g.
Urea	20.0 g.

Cunningham and Löhnis used the following basic solution to study the assimilation of ammoniacal and nitrite nitrogen.

1. Distilled water	1000.0 cc.
2. Glycerol	10.0 g.
3. K_2HPO_4	0.5 g.
4. $MgSO_4$	0.02 g.
5. NaCl	0.02 g.
6. Fe_2Cl_6	trace

Cunningham added 0.1% of $(NH_4)_2SO_4$ or 0.1% of $NaNO_2$.

Löhnis added 0.1% of urea sodium hippurate, uric acid, asparagin, ammonium sulphate acetate, butyrate lactate or $NaNO_3$. Löhnis also specified that other carbohydrates, alcohols and acids might be used instead of glycerol.

Reference: Gerlach and Vogel (1901 p. 612). Löhnis (1913 p. 94), Cunningham (1924 p. 153).

146. Kuhne's Basal Lactate Solution (Proskauer and Beck)

Constituents:

1.	Water	600.0 cc.
2.	$MgSO_4$	3.5 g.
3.	Gypsum (burned)	1.5 g.
4.	NaCl	16.0 g.
5.	Magnesium (burned)	2.5 g.
6.	Potash (dried)	62.13 g.
7.	Soda	7.35 g.
8.	Ferrum reductum	6.2 g.
9.	H_3PO_4 (Sp. G. 1.3)	95.0 g.
10.	Lactic acid (Sp. G. 1.2)	50.0 to 60.0 g.
11.	Glycerol	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8, 9 and 10 in 1.

(2) Add 12.0 cc. of (1), 40.0 g. glycerol, 5.0 g. NaCl and 1000.0 cc. of water to one of the combinations listed under added nutrients.

Sterilization: Not specified.

Use: To study the requirements for the growth of tubercle bacilli. Growth generally occurred. The authors stated that 12.0 cc. of the basic solution was the equivalent of 10.0 g. of commercial Liebig's meat extract.

Added nutrients: The authors use 12.0 cc. of (1) basic salt solution and add 1000.0 cc. water, 40.0 g. glycerol and 5.0 g. of NaCl. The following materials were added to this solution:

(a)	tyrosine	10.0 g.
	asparagin	2.0 g.
	ammonium mucate	2.0 g.
	taurine	0.5 g.
(b)	leucine	4.0 g.
	asparagin	2.0 g.
	ammonium mucate	2.0 g.
	taurine	0.5 g.
(c)	tyrosine	1.0 g.
	ammonium mucate	2.0 g.
	taurine	0.5 g.
(d)	tyrosine	1.0 g.
	asparagin	2.0 g.
	taurine	0.5 g.
(e)	tyrosine	1.0 g.
	asparagin	2.0 g.
	ammonium mucate	2.0 g.
(f)	leucine	4.0 g.
	tyrosine	1.0 g.
	asparagin	3.0 g.
(g)	leucine	4.0 g.
	tyrosine	1.0 g.
	ammonium mucate	2.0 g.
(h)	leucine	4.0 g.
	asparagin	2.0 g.
	ammonium mucate	2.0 g.
(i)	tyrosine	1.0 g.
	asparagin	2.0 g.
	ammonium mucate	2.0 g.
(j)	leucine	4.0 g.
	tyrosine	1.0 g.
	taurine	0.5 g.
(k)	leucine	4.0 g.
	asparagin	2.0 g.
	taurine	0.5 g.
(l)	tyrosine	1.0 g.
	asparagin	2.0 g.
	taurine	0.5 g.
(m)	leucine	4.0 g.
	ammonium mucate	2.0 g.
	taurine	0.5 g.
(n)	tyrosine	1.0 g.
	ammonium mucate	2.0 g.
	taurine	0.5 g.
(o)	asparagin	2.0 g.
	ammonium mucate	2.0 g.
	taurine	0.5 g.
(p)	leucine	4.0 g.
	tyrosine	1.0 g.
	asparagin	2.0 g.
	ammonium mucate	2.0 g.
	taurine	0.5 g.
(q)	alanine	4.0 g.
	asparagin	2.0 g.
(r)	alanine	4.0 g.
	asparagin	3.0 g.
	ammonium mucate	3.0 g.

(s) Used 7.35 soda instead of 6.35 g. in the basal solution and added:

leucine	4.0 g.
alanine	4.0 g.
glycocoll	4.0 g.
glucose	2.0 g.

(t) glycocoll	3.0 g.
glycerol	40.0 g.
taurine	5.0 g.

(u) Tomasczewski used 7.35 soda in the basal solution and added:

tyrosine	1.0 g.
leucine	4.0 g.
asparagin	2.0 g.
ammonium mucate	3.0 g.
taurine	0.5 g.

Reference: Proskauer and Beck (1894 p. 129–131, 134–135), Tomasczewski (1899 p. 249).

147. Calimard and Lacommes' Basal Glycerol Salt Solution (Kolle and Wassermann)

Constituents:

1. Water	1000.0 cc.
2. NaCl (0.5%)	5.0 g.
3. $MgSO_4$ (0.05%)	0.5 g.
4. Glycerol potassium phosphate (0.2%)	2.0 g.
5. Glycerol (1.5%)	15.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and one of the added nutrients in 1.

Sterilization: Not specified.

Use: General culture medium.

Added nutrients: The author added one of the following:

urea, amines, leucine, tyrosine, etc., etc.

Reference: Kolle and Wassermann (1912 p. 394).

148. Beijerinck's Modified Mannitol Salt Solution (Omeliansky and Sswerowa)

Constituents:

1. Distilled water	1000.0 cc.
2. Mannitol	20.0 g.
3. Potassium phosphate	1.0 g.
4. $MgSO_4$	0.5 g.
5. NaCl	0.1 g.
6. Iron sulphate	0.1 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not given.

Use: To study the pigment production of *Azotobacter chroococcum.*

Variants: Harvey used the following variant of this solution to study nitrification:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $MgSO_4$	0.2 g.
4. NaCl	0.02 g.
5. $MnSO_4$ (1:1000)	1.0 cc.
6. $FeSO_4$ (1:1000)	1.0 cc.
7. Mannitol	20.0 g.

Reference: Omeliansky and Sswerowa (1911 p. 645), Harvey (1921–22 p. 105).

149. Murray's Mannitol Salt Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	0.2 g.
3. $MgSO_4$	0.2 g.
4. NaCl	0.5 g.
5. Mannite	20.0 g.
6. $FeCl_2$	1 drop of 10% sol.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Neutralize with KOH using phenolphthalein as indicator.

Sterilization: Not specified.

Use: To study the fixation of nitrogen under anaerobic and aerobic conditions by bacteria from the soil.

Reference: Murray (1916 p. 604).

150. Percival's Mannitol Phosphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4	2.0 g.
3. Magnesium phosphate	1.0 g.
4. Mannitol	20.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add 4 to (1).

(3) Heat to boiling.

(4) Filter.

(5) Adjustment of reaction not specified.

(6) Tube in 10 to 15.0 cc. quantities.

(7) After sterilization inoculate with contents of a nodule of a pea, runner bean, red clover, or broad bean, by means of a sterile knife.

Sterilization: Sterilize on 3 successive days (time not given).

Use: Cultivation of bacteria from the roots of leguminous plants (bacteroids).

Reference: Percival (1920 p. 204).

151. Capaldi and Proskauer's Basal Mannitol Salt Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl (0.02%)	0.2 g.
3. $MgSO_4$ (0.01%)	0.1 g.
4. KH_2PO_4 (0.2%)	2.0 g.
5. Mannitol (0.2%)	20.0 g.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Add a suitable nitrogen source.

Sterilization: Not specified.

Use: To study acid production by *B. coli*.

Added nutrients: The authors added one of the following nitrogen sources:

glycocoll
alanine
leucine
oxamide
succinamide
tyrosine
urea
sulfocarbamid
guanidinchlorhydrate
guanine
creatine
sarcosine
allantoin
alloxantin
alloxan
theobromine
caffein
potassium urate
glycocoll + urea
ammonium formate
ammonium acetate
ammonium butyrate
ammonium isobutyrate
ammonium valerate
ammonium stearate
ammonium oleate
ammonium lactate
ammonium pyroracemate
ammonium oxalate
ammonium malonate
ammonium succinate
ammonium tartrate
ammonium fumarate
ammonium mucate
ammonium citrate
ammonium aconitate
ammonium itaconate
ammonium citraconate
ammonium amidobenzoate
ammonium hippurate
ammonium orthonitrozimmtate
ammonium piperate
ammonium carbamate
ammonium cyanurate
ammonium carbonate

Reference: Capaldi and Proskauer (1896 p. 456).

152. Percival's Succinate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4	2.0 g.
3. Magnesium phosphate	0.1 g.
4. Sodium succinate	5.0 g.

Preparation:
(1) Dissolve 2 and 3 in 1.
(2) Add 4 to (1).
(3) Heat to boiling.
(4) Filter.
(5) Adjustment of reaction not specified.
(6) Tube in 10 to 15.0 cc. quantities.
(7) After sterilization inoculate with contents of a nodule of a pea, runner bean, red clover, or broad bean by means of a sterile knife.

Sterilization: Sterilize on 3 successive days (time not given).

Use: Cultivation of bacteria from the roots of leguminous plants, (bacteroids).

Reference: Percival (1920 p. 204).

153. Winogradsky's Glucose Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Glucose	20.0 g.
3. Potassium phosphate	1.0 g.
4. $MgSO_4$	0.2 g.
5. NaCl	small amount
6. $FeSO_4$	small amount
7. $MnSO_4$	small amount
8. Chalk	

Preparation:
(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Adjustment of reaction not specified.
(3) Distribute into Drechsel's flasks with a ground cover.
(4) Add an excess of chalk to each flask.

Sterilization: In the autoclave.

Use: Used by Winogradsky to study nitrogen fixation by *Clostridium Pastorianum*. Other investigators used other nitrogen fixing bacteria.

Variants:
(a) von Freudenreich used 20 to 40.0 g. glucose, 0.5 g. of potassium phosphate instead of 0.2 g., and specified 0.01 to 0.02 g. of NaCl, $FeSO_4$ and $MnSO_4$.
(b) Jacobitz used the same variation as (a) but specified distilled water.
(c) Smith used 20 to 40.0 g. glucose, 0.5 g. $MgSO_4$, 0.01 to 0.02 g. NaCl, 0.1 to 0.2 g. iron sulphate, instead of

$FeSO_4$, omitted the $MnSO_4$ and used 30.0 to 40.0 g. $CaCO_3$ per liter.

(d) Bredemann specified 1.0 g. K_2HPO_4, 0.02 g. NaCl, 0.1 g. $FeSO_4$, and 0.01 g. $MnSO_4$ but did not specify distilled water.
(e) Percival specified 1.0 g. K_2HPO_4, used 30.0 g. glucose and 0.5 g. $MgSO_4$.
(f) Lantzsch specified 1.0 g. K_2HPO_4, 0.01 g. NaCl, 0.01 g. $FeSO_4$ and omitted the $MnSO_4$. He did not specify distilled water.
(g) Lantzsch specified the use of 0.01 g. NaCl, 0.2 g. $FeSO_4$ used 50.0 g. $CaCO_3$, and omitted the $MnSO_4$. This solution was employed as a basic solution and added one of the following to each 100.0 cc. of solution:
  (a) 2.0, 5.0 or 10.0 cc. of a 2.0% NaOH (soil extract).
  (b) 0.28 g. or 0.56 g. animal charcoal.
  (c) 0.4 g., 1.0 g. or 3.0 g. of Bolus alba (pure kaolin).
  (d) 0.25% or 1.0% gelatin.

This medium was used for the cultivation of amylobacter spores.

References: Winogradsky (1902 p. 49), von Freudenreich (1903 p. 516), Jacobitz (1903 p. 100), Smith (1905 p. 199), Bredemann (1909 p. 496), Percival (1920 p. 174), Lantzsch (1921 p. 2), Lantzsch (1921 p. 5).

154. Stutzer, Burri and Maul's Glucose Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Asbestos	
3. Potassium phosphate	1.0 g.
4. $MgSO_4$	0.5 g.
5. NaCl	1.0 g.
6. $CaCl_2$	0.1 g.
7. Dextrose	20.0 g.

Preparation:

(1) Dissolve 3, 4, 5, 6 and 7 in 1.
(2) Heat long fibre asbestos to glowing.
(3) To a 2 liter flask add 10.0 g. (2) 25.0 cc. (1), 25.0 cc. sterile water.
(4) After sterilization add a large platinum loop of a gelatin streak culture of an organism to 100.0 cc. sterile water. Add 2.0 cc. of (5) to (4).

Sterilization: Method not given.

Use: To determine nitrogen fixation. Authors used the bacteria from mustard and alfalfa plant nodules and found that little, if any, nitrogen was assimilated after 60 days incubation at 28 to 30°C.

Reference: Stutzer, Burri and Maul (1896 p. 669).

155. Stoklasa's Basal Salt Solution

Constituents:

1. Water	2000.0 cc.
2. Sodium phosphate	0.5 g.
3. K_2SO_4	0.2 g.
4. $MgCl_2$	0.05 g.
5. Glucose	2.5 g.
6. $CaCO_3$	10.0 g.

Preparation:

(1) Dissolve 0.5 g. sodium phosphate, 0.2 g. potassium sulphate and 0.05 g. magnesium chloride in 500.0 cc. of water.
(2) Dissolve 2.5 g. glucose in 500.0 cc. water.
(3) Mix 200.0 cc. (1) with 100.0 cc. (2).
(4) Dissolve 6 and one of the added nutrients in (3).
(5) Dilute to 2000.0 cc.

Sterilization: Method not given.

Use: To study utilization of nitrogen by *Bacillus megatherium*, (*Bacillus Ellenbachii*) or "Alinit." The author reported that more nitrogen was found to be in solution after incubation. These organisms decomposed nitrogenous material to a form that may be absorbed by the root hairs.

Added nutrients: The author added 50.0 g. of glutin or fibrin.

Reference: Stoklasa (1898 p. 508).

156. Charpentier's Glucose Salt Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	0.25 g.
3. KH_2PO_4	0.25 g.
4. $MgSO_4$	0.37 g.
5. Iron phosphate	trace
6. $CaSO_4$	trace
7. NaCl	0.2 g.
8. Glucose	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Distribute in flasks.
(3) Add 0.075 g. glucose to each flask. Amount of medium in the flask not specified.

(4) Pass a current of air thru H_2SO_4 and then thru the culture.

Sterilization: Not specified.

Use: Nitrogen fixation by algae; *Cystococcus humicola* did not fix free nitrogen.

Reference: Charpentier (1903 p. 322).

157. Krzemieniewska's Glucose Salt Solution (Vogel)

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4 \cdot 7H_2O$	0.25 g.
3. $CaHPO_4$	0.25 g.
4. Glucose	13.5 g.
5. K_2SO_4	0.0 to 0.872 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add from 0.0 to 87.2 mg. of K_2SO_4 to (1).

Sterilization: Not specified.

Use: Nitrogen fixation. The author found that the most nitrogen was fixed in the presence of 0.872 g. K_2SO_4.

Reference: Vogel (1911–12 p. 417).

158. Krüger and Schneidewind's Basal Glucose Salt Solution (Heinze)

Constituents:

1. Water	1000.0 cc.
2. Glucose (1.0%)	10.0 g.
3. K_3PO_4 (0.2%)	2.0 g.
4. $MgSO_4$ (0.04%)	0.4 g.
5. $CaCl_2$ (0.02%)	0.2 g.
6. $FeCl_3$ (20.0% soln.)	1 drop

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Add one of the nitrogen sources listed in added nutrients.

Sterilization: Not specified.

Use: To study nitrogen assimilation by green algae, *Chlorella*, *Chlorothecium*, *Stichococcus*. The author reported that nitrogen was not assimilated. Poor growth unless nitrogen was present.

Variants: The solution may be used without the addition of any nitrogen source.

Added nutrients: The author added 0.25% of $(NH_4)_2SO_4$ or $NaNO_3$.

Reference: Heinze (1906 p. 647).

159. Charpentier's Basal Glucose Salt Solution

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4$	1.0 g.
3. K_2HPO_4	2.0 g.
4. $CaCl_2$	0.1 g.
5. $FeSO_4$	0.05 g.
6. Glucose	10.0 g.

Preparation:

(1) Dissolve one of the added nutrients in water.

(2) Dissolve 2, 3, 4, 5 and 6 in the remainder of the water.

(3) Mix sterile (1) and (2) under aseptic conditions.

Sterilization: Sterilize (1) by filtration and (2) in the autoclave.

Use: To study assimilation of nitrogen by algae, *Cystococcus humicola*. Author reported that $Ca(NO_3)_2$ was a more suitable nitrogen source than asparagin or peptone.

Added nutrients and variants:

(a) The author added 1.8 g. of peptone or 1.8 g. asparagin as a nitrogen source.

(b) Müller omitted the $FeSO_4$, used 0.1% K_2HPO_4 and 0.2% $MgSO_4$ in the basic solution and added one of the following nitrogen sources. He cultivated *Bacterium Güntheri*.

KNO_3
ammonium tartrate
asparagin
peptone

References: Charpentier (1903 p. 333), Müller (1907 p. 472).

160. Stoklasa's Basal Glucose Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. d-glucose	25.0 g.
3. Phosphoric acid anhydride	0.2 g.
4. K_2SO_4	1.0 g.
5. $MgCl_2$	0.5 g.
6. Iron sulphate	+
7. Aluminum sulphate	0.1 g.
8. $CaCl_2$	0.1 g.

Preparation:

(1) Dissolve 2, 4, 5, 6, 7 and 8 in 1.

(2) Add one of the added nutrients to (1).

(3) Add 0.2 g. of phosphoric acid anhydride in the form of monomagnesium phosphate, monodialuminum phosphate, monodiferric phosphate or trimagnesium phosphate to (2).

(4) Inoculate 8 days following sterilization.

Sterlization: In the autoclave.

Use: To study rôle of phosphorus in the soil. Development of the bacteria hardly visible when phosphoric anhydride was omitted.

Added nutrients: The author added 1.0 g. nitrogen in the form of $(NH_4)_2SO_4$ or $(NH_4)NO_3$.

Reference: Stoklasa (1911 p. 490).

161. Henneberg's Basal Glucose Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4 (0.1%)	1.0 g.
3. $MgSO_4$ (0.02%)	0.2 g.
4. $CaCl_2$ (0.01%)	0.1 g.
5. Glucose (2.0%)	20.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 1.0% of one of the added nutrients in 1.

Sterilization: Not specified.

Use: To study nitrogen requirements for lactic acid bacteria. Urea, asparagin or ammonium phosphate were found not to be suitable as nitrogen sources.

Added nutrients: The author added 1.0% of one of the following:

peptone
casein
gluten
asparagin
urea
ammonium phosphate

Reference: Henneberg (1903 p. 7).

162. Gottheil's Carbohydrate Glycerol Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.3 g.
5. NaCl	0.1 g.
6. Iron	trace
7. Dextrose	5.0 g.
8. Cane sugar	5.0 g.
9. Glycerin	5.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

Sterilization: Not specified.

Use: Used as a general culture medium. Author used bacteria found in the soil and on the roots of plants.

Reference: Gottheil (1901 p. 432).

163. Harvey's Citric Acid Glucose Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Glucose	2.0 g.
3. Citric acid	5.0 g.
4. $MgSO_4$	2.0 g.
5. K_2HPO_4	2.0 g.
6. $CaCl_2$	0.2 g.
7. $FeCl_3$	trace

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in distilled water.

(2) Reaction should be neutral (indicator not specified).

Sterilization: Not given.

Use: Nitrogen fixation by aerobic nitrogen fixing bacteria.

Reference: Harvey (1921–22 p. 104).

164. Münter's Basal Mannitol Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Mannitol	2.0 g.
3. Glucose	10.0 g.
4. $MgSO_4$	0.5 g.
5. NaCl	0.5 g.
6. K_2HPO_4	2.0 g.
7. $CaCl_2$	0.1 g.
8. $FeCl_3$	trace

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

(2) Add 0.02 or 0.1 g. of nitrogen in the form of one of the added nutrients.

(3) Adjustment of reaction not given.

(4) Distribute in 100.0 cc. lots.

Sterilization: Not specified.

Use: To study availability of nitrogen for *Actinomycetes odorifer*, *Act. chromogenes*, *Act. albus*. NH_4Cl, NH_4NO_3 and asparagin were found to be equally suitable nitrogen sources for the actinomycetes studied.

Added nutrients: The author used the following nitrogen sources:

NH_4Cl
NH_4NO_3
asparagin

Reference: Münter (1913 p. 368).

165. Chrzaszcz's Basal Sucrose Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Sucrose	100.0 g.
3. $MgSO_4$	2.0 g.
4. K_2SO_4	2.0 g.
5. Na_2HPO_4	5.0 g.
6. $CaCO_3$	1.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Add one of the added nutrients to (1).

Sterilization: Not specified.

Use: To study growth requirements of yeasts. Author reported that yeast grew more luxuriantly in a medium containing peptone than asparagin. Growth best, however, in sugar beer wort medium.

Added nutrients: The author added 5.0 g. of asparagin or 5.0 g. of peptone.

Reference: Chrzaszcz (1904 p. 148).

166. Mazé's Sucrose Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Sucrose	20.0 g.
3. Potassium phosphate	1.0 g.
4. NaCl	1.0 g.
5. $FeSO_4$	trace
6. $MgSO_4$	trace
7. $ZnCl_2$	trace
8. Calcium saccharate	trace

Preparation:
(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.
(2) Adjustment of reaction not specified.

Sterilization: Sterilize at 120°C. (time not given).

Use: Cultivation of bacteria from the nodules of leguminous plants, nitrogen fixing bacteria.

Reference: Mazé (1898 p. 12).

167. Linde's Basal Sucrose Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $MgSO_4 \cdot 7H_2O$	0.3 g.
4. NaCl	0.1 g.
5. $CaCl_2$	0.1 g.
6. Sucrose (0.2%)	2.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Add 0.15% of one of the nitrogen sources given in added nutrients.

Sterilization: Not specified.

Use: Cultivation of Cladothrix. The author reported that the organism grew better on meat extract media.

Added nutrients: The author added 0.15% of one of the following:

KNO_3
NH_4Cl
$(NH_4)_3PO_4$
$(NH_4)_2SO_4$

Reference: Linde (1913 p. 387).

168. Löhnis and Lochhead's Basal Cellulose Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Basic slag ("Thomasmehl") (0.2%)	2.0 g.
3. K_2HPO_4 (0.02%)	0.2 g.
4. $MgSO_4$ (0.01%)	0.1 g.
5. NaCl (0.001%)	0.01 g.
6. Filter paper.	

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Add one of the nitrogen sources listed under added nutrients.
(3) The reaction is slightly alkaline, but may be neutralized by HCl or lactic acid.
(4) Place strips of filter paper in flasks.
(5) Pour about 10.0 cc. of (2) over the paper.
(6) Inoculate with about 1.0 g. of soil or cow manure.

Sterilization: Not specified.

Use: Cultivation of cellulose dissolving bacteria. Author found that the best growth was obtained on medium containing 0.1% beef extract. KNO_3 was the best inorganic nitrogen source. The solution was carefully poured from the filter paper as soon as it has become turbid by bacterial growth and fresh solution added.

Added nutrients: The author used the following nitrogen sources in the amounts indicated:

Peptone	0.1 or 0.02%
Beef extract	0.1 or 0.02%
Asparagin	0.25 or 0.05%
$(NH_4)_2SO_4$	0.5 or 0.1%
NH_4NO_3	0.25 or 0.05%
KNO_3	0.5 or 0.1%

Reference: Löhnis and Lochhead (1923 p. 431).

169. van Iterson's Basal Cellulose Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Paper	20.0 g.
3. K_2HPO_4	0.5 g.

Preparation:
(1) Mix 1, 2 and 3.
(2) Add one of the nitrogen sources given in added nutrients, to (1).

(3) When using KNO_3 distribute by filling 200.0 cc. flasks. When using other nitrogen sources distribute in thin layers in flasks.
(4) Inoculate with canal water containing some mold or decay (moder).

Sterilization: Not specified.

Use: To study the decomposition of cellulose by denitrifying organisms. The author reported denitrification and decomposition of cellulose with the production of N_2 and CO_2.

Added nutrients: The author used the following nitrogen sources:

KNO_3	2.5 g.
NH_4Cl	1.0 g.

KNO_2 amount not given
$MgNH_4PO_4$ amount not given
peptone amount not given

Variants: The author sprinkled $MgNH_4PO_4$ between two Swedish filter papers and placed them in a flask. Then he poured a solution of 0.5 g. K_2HPO_4 in a liter of water over the paper. He found that *Bacillus ferrugineus* from the soil decomposed the cellulose.

Reference: van Iterson (1903–4 p. 690).

170. Jones' Modified Ashby's Mannitol Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Mannitol	20.0 g.
3. K_2HPO_4	0.2 g.
4. $MgSO_4$	0.2 g.
5. NaCl	0.2 g.
6. $CaSO_4$	0.1 g.
7. $CaCl_3$	5.0 g

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Cultivation of *Azotobacter*.

Variants:
(a) Bonazzi specified the use of tap water, used 0.408 g. $MgSO_4 \cdot 7H_2O$; 0.127 g. $CaSO_4$; 0.2 g. $CaCO_3$ and specified that $Ca(NO_3)_2$ might be added if desired (amount not given).
(b) Giltner used 15.0 g. of mannitol and added 1 drop of 10.0% Fe_2Cl_6 solution.

Reference: Jones (1913 p. 14), Bonazzi (1921 p. 357), Giltner (1921 p. 376).

171. Fred and Loomis' Mannitol Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Mannitol	10.0 g.
3. $MgSO_4$	0.2 g.
4. KH_2PO_4	0.2 g.
5. NaCl	0.2 g.
6. $CaSO_4$	0.1 g.

Preparation:
(1) Dissolve 2, 3, 5 and 6 in 1.
(2) Dissolve phosphate separately in a little water and add to (1).
(3) Make (2) neutral to phenolphthalein with N/1 NaOH.
(4) After sterilization add NaOH and H_2SO_4 to obtain pH value from 2.7 to 11.1.

Sterilization: Method not specified.

Use: To show the effect of hydrogen ion concentration on growth of Rhizobium. Maximum growth at pH 7.2. None in pH 2.77 and 3.1. Yamagata and Itano found the optimum pH for azotobacter to be between 6.6 and 7.7 depending on the type of azotobacter.

Variants: Yamagata and Itano used 20.0 g. of mannitol instead of 10.0 g.

Reference: Fred and Loomis (1917 p. 629), Yamagata and Itano (1923 p. 522).

172. Krzemieniewska's Mannitol Salt Solution (Vogel)

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	2.5 g.
3. $MgSO_4$	2.5 g.
4. $CaSO_4$	2.5 g.
5. Mannitol	20.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of *Azotobacter*.

Variants: The author used 2.5 g. $CaHPO_4$ instead of 2.5 g. $CaSO_4$, or omitted the K_2HPO_4 and added 2.5 g. $CaHPO_4$ instead of $CaSO_4$.

Reference: Vogel (1911–12 p. 416).

173. Harvey's Mannitol Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Mannitol	15.0 g.
3. $MgSO_4$	0.2 g.
4. KH_2PO_4	0.2 g.

5. NaCl	0.2 g.
6. $CaSO_4$	0.1 g.
7. $CaCO_3$	5.0 g.

Preparation:

(1) Dissolve 4 in a little distilled water.

(2) Neutralize (1) to phenolphthalein.

(3) Add 2, 3, 5, 6 and 7 to (2) and make up to 1000.0 cc.

Sterilization: Not specified.

Use: Cultivation of aerobic nitrogen fixing bacteria and protozoa.

Reference: Harvey (1921–22 p. 104).

174. Sackett's Modified Lipman's Mannitol Salt Solution

Constituents:

1. Water (surface)	1000.0 cc.
2. Mannitol	15.0 g.
3. K_2PO_4	0.5 g.
4. $MgSO_4$	0.2 g.
5. $CaCl_2$	0.02 g.
6. 10.0% $FeCl_3$ solution	1 drop

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1000.0 cc. of surface water.

(2) Neutralize with N/1 NaOH to phenolphthalein.

(3) Distribute in 100.0 cc. lots in 500.0 cc. Erlenmeyer flasks.

Sterilization: Autoclave for 5 minutes at 120°C.

Use: Study nitrogen fixation by bacteria found in soil.

Variants: Heinemann used 0.2 g. K_2HPO_4 instead of 0.5 g. K_3HPO_4 and 0.01 g. Fe_2Cl_6.

Reference: Sackett (1912 p. 87), Heinemann (1922 p. 39).

175. Winogradsky's Mannitol Salt Solution (Lantzsch)

Constituents:

1. Water	1000.0 cc.
2. Mannitol	20.0 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4$	0.2 g.
5. $FeSO_4$	0.01 g.
6. NaCl	0.01 g.
7. $CaCO_3$	5.0 g.

Preparation:

(1) Dissolve one of 2, 3, 4, 5, 6 and 7 in 1.

(2) To prepare anaerobic culture after inoculation, stopper the flasks with 2 holed stoppers, seal with paraffin and pass N_2 that has been washed in potassium pyrogallic acid solution three times, then in H_2SO_4 and NaOH, thru the flasks.

Sterilization: Not specified.

Use: To study nitrogen fixation by *Bacillus amylobacter*, *Bact. pneumoniae* and torula under anaerobic and aerobic conditions.

Variants: The author suggests the use of glucose instead of mannitol and specifies that 10.0 or 20.0 g. of calcium humate may or may not be added to the glucose medium.

Reference: Lantzsch (1921 p. 2).

176. Koser and Rettger's Basal Glycerol Salt Solution

Constituents:

1. Distilled NH_3 free water	1000.0 cc.
2. NaCl	5.0 g.
3. $MgSO_4$	0.2 g.
4. $CaCl_2$	0.1 g.
5. KH_2PO_4	1.0 g.
6. K_2HPO_4	1.0 g.
7. Glycerol	30.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Add one of the added nutrients to (1).

(3) pH = 6.6.

(4) Medium is clear and colorless and requires no filtration.

(5) Distribute into test tubes.

Sterilization: Autoclave at 10 to 12 pounds pressure for 15 minutes.

Use: To study bacterial nutrition.

Added nutrients: The authors used 1.0 g. of one of the following:

valin
glutamic acid
tyrosin
phenylalanin
lysin picrate
urea
uric acid
hypoxanthin
allantoin
creatin
glycocoll
aspartic acid
asparagin
tryptophan
leucin
histidin monochloride

lysine dichloride
taurine

Variants: The authors found that combinations of amino acids possessed little value over single amino acids.

Reference: Koser and Rettger (1919 p. 304), Koser (1918, p. 12).

177. Puriewitsch's Tartaric Acid Salt Solution (Heinze)

Constituents:

1. Water	1000.0 cc
2. Tartaric acid	3.0 g.
3. $MgSO_4$	0.2 g.
4. $CaCl_2$	0.4 g.
5. KH_2PO_4	0.4 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Study of nitrogen assimilation by molds, *Aspergillus niger* and *Penicillum glaucum*.

Reference: Puriewitsch (1895 p. 342 from an abstract by Heinze 1903 p. 27).

178. Omeliansky's Cellulose Solution

Constituents:

1. Water
2. Chalk
3. Paper.

Preparation: (1) Inoculate a flask of water containing chalk and paper with some river mud.

Use: To study cellulose fermentation by organisms found in mud of rivers. The paper was completely destroyed after 3.5 years.

Reference: Omeliansky (190 p. 229).

179. Killer's Mannitol Solution

Constituents:

1. Water	1000.0 cc.
2. Mannitol	20.0 g.
3. Potassium phosphate	0.5 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: To study nitrogen assimilation by soil bacteria and protozoa.

Variants: Dilute the medium 5 to 10 times for cultivation of protozoa.

Reference: Killer (1913 p. 522).

180. Ringer's Salt Solution (Park, Williams and Krumwiede)

Constituents:

1. Water	1000.0 cc.
2. NaCl	10.0 g.
3. KCl	0.2 g.
4. $CaCl_2$	0.2 g.
5. Sodium bicarbonate	0.1 g.
6. Glucose	1.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Diluent and a basis solution to which carbonaceous and nitrogenous material may be added. (See medium 600.)

Reference: Park, Williams and Krumwiede (1924 p. 122).

181. Beijerinck's Glucose Salt Solution

Constituents:

1. Water (ditch)	1000.0 cc.
2. Glucose	0.05 g.
3. Potassium phosphate	0.1 g.
4. $FeCl_3$	several drops
5. Na_2CO_3	0.5 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in ditch water ("Grabenwasser") containing 45 mg. SO_3 per liter.

(2) Distribute into flasks or beaker. Cover the beaker with a glass plate or fill the flask full to reduce the surface exposed to the air.

Sterilization: Not specified.

Use: To study sulphate reduction by *Spirillum desulfuricans*. H_2S and H_2SO_3 can be detected after 12 hours.

Reference: Beijerinck (1895 p. 57).

182. Stoklasa's Glucose Salt Solution

Constituents:

1. River water	1000.0 cc.
2. Glucose	20.0 g.
3. K_2HPO_4	0.5 g.
4. Na_2CO_3	0.25 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Adjustment of reaction not given.

(3) Distribute in thin layers in large flasks.

Sterilization: Sterilize thoroughly in the autoclave.

Use: To study the utilization of carbohydrate and nitrogen assimilation by *Azotobacter chroococcum* Beijerinck.

Reference: Stoklasa (1908 p. 503).

183. Gage's Maltose Salt Solution

Constituents:

1. Water nitrite free	1000.0 cc.
2. KH_2PO_4	1.0 g.

3. $NaCl$	0.5 g.
4. Maltose	1.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1000.0 g. of nitrite free water.

(2) Distribute in 100.0 cc. lots in 150.0 cc. flasks.

Sterilization: Not specified.

Use: To study the fixation of nitrogen by nitroso bacteria. No nitrogen was fixed by the culture studied.

Reference: Gage (1910 p. 19).

184. Söhngen's Colloid Mannitol Solution

Constituents:

1. Water	1000.0 cc.
2. Mannitol	20.0 g.
3. K_2HPO_4	0.5 g.
4. Iron aluminum silicon about	0.1 g.
5. Colloidal material	

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) After sterilization add one of the following sterile materials to 100.0 cc. of (1).

(a) 2.0 g. raw humus (prepared by sieving and washing soil, moisten with several drops of soda solution).

(b) 400 mg. of humus acid containing traces of iron, aluminum and silicon.

(c) 500 mg. colloidal silicon dioxide as a sol.

(d) 400 mg. Thompson phosphate.

(e) 200 mg. purified potassium humate.

(f) 500 mg. of iron oxide as a sol.

Sterilization: Method not given.

Use: To study the effect of colloids on bacterial processes (nitrogen assimilation) by azotobacter. The most nitrogen was assimilated in the presence of raw humus and silicon dioxide.

Reference: Söhngen (1913 p. 627).

185. Beijerinck's Phosphate Glucose Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose	20.0 g.
3. K_2HPO_4	0.2 g.
4. $CaCO_3$	20.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add 4 to (1).

Sterilization: Not specified.

Use: Growth of mesonitrophilic organisms, *Azotobacter agilis*, *Granulobacter sphericum*. When inoculated with pasteurized garden soil after 2 or 3 days a fermentation takes place. The odor of ethyl or propyl alcohol is given off.

Variants: (1) Bonazzi specified the use of deep well water and added 0.5 g. $CaCO_3$ for each 100.0 cc. of medium. He also specified that 1.246 g. $Ca(NO_3)_2$ might be added if desired.

Reference: Beijerinck (1901 p. 573), Bonazzi (1921 p. 336).

186. Buhlert and Fickendey's Phosphate Glucose Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. Glucose	20.0 g.
4. $NaCl$	1.0 g.
5. $CaCO_3$	4.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Distribute in large Erlenmeyer flasks.

Sterilization: Not specified.

Use: To study nitrogen fixation by bacteria from the soil.

Variants:

(a) Barthel used 10.0 g. $CaCO_3$.

(b) Percival used 5.0 g. $CaCO_3$.

References: Buhlert and Fickendey (1906 p. 403), Barthel (1910 p. 122), Percival (1920 p. 209).

187. Wojtkiewicz's Mannitol Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Mannitol	20.0 g.
3. K_2HPO_4	0.2 g.
4. Chalk	5.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Distribute in 125.0 cc. lots in 500.0 cc. Erlenmeyer flasks.

Sterilization: Not specified.

Use: To study nitrogen assimilation by bacteria from the soil.

Variants:

(a) Hanzawa used 0.5 g. K_2HPO_4 and did not specify the amount of chalk to be added.

(b) Hanzawa suggested the use of soil

extract instead of water in variant (a). Nitrogen assimilation was better in plain solution however.

(c) Hanzawa suggested the addition of humus or saltpeter in varying amounts to variant (a).

References: Wojtkiewicz (1914 p. 255), Hanzawa (1914 p. 574).

188. Krainsky's Mannitol Salt Solution

Constituents:

1.	Water	1000.0 cc.
2.	KH_2PO_4	0.5 g.
3.	NaCl	0.5 g.
4.	$CaCO_3$	0.5 g.
5.	$FeSO_4$	trace
6.	Mannite	10.0 g. to 20.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Enrichment of *Azotobacter chroococcum.*

Reference: Krainsky (1908 p. 726).

189. Peklo's Modified Beijerinck's Mannitol Salt Solution

Constituents:

1.	Water (river)	1000.0 cc.
2.	Mannitol	25.0 g.
3.	K_2HPO_4	8.0 g.
4.	K_2CO_3	4.0 g.
5.	$CaCO_3$	

Preparation:

(1) Dissolve 2, 3 and 4 in Moldau river water.

(2) Add $CaCO_3$, amount not specified.

Sterilization: Not specified.

Use: Cultivation of plant actinomyces.

Reference: Peklo (1910 p. 514).

190. Butcher's Basal Chinate Salt Solution

Constituents:

1.	Water	1000.0 cc.
2.	K_2HPO_4	0.5 g.
3.	Calcium chinate	10.0 g.
4.	$FeCl_3$	0.1 g.

Preparation: (1) Dissolve 2, 3, 4 and one of the added nutrients in 1.

Sterilization: Not specified.

Use: Differentiation of colon-aerogenes group. Author reported that a solid medium was preferred to a liquid medium. *Bact. aerogenes* gave a black coloration to the medium, while *Bact. coli* did not.

Added nutrients: The author added 0.5 g. NH_4Cl or 0.5 g. peptone.

Reference: Butcher (1924 p. 295).

SUBGROUP I-C. SECTION 2

Liquid media or basal solutions with constituents of known chemical composition; nitrogen supplied as ammonium salts, carbon organic.

A_1.* Media primarily for growth of yeast, fungi, or algae; not bacteria.
B_1. Ammonia supplied as ammonium chloride.
  Wilder's Sugar Salt Solution (Chrzaszcz) ... 191
  LaGarde's Sucrose Ammonium Chloride Solution ... 192
  Fulmer and Nelson's Sucrose Ammonium Chloride Solutions ... 193
B_2.† Ammonia Supplied as ammonium nitrate.
C_1. Organic carbon supplied only as carbohydrates.
D_1. Mono or disaccharides added.
E_1. Calcium salts added.
  Mayer's Sucrose Ammonium Nitrate Solution (Pringsheim) ... 194
  Bokorny's' Sucrose Ammonium Nitrate Solution ... 195
E_2. Calcium salts not added.
  Hollborn's Sucrose Ammonium Nitrate Solution ... 196
  Laborde's Sucrose Ammonium Nitrate Solution ... 197
  Ono's Sucrose Ammonium Nitrate Solution ... 198
D_2. Polysaccharides added.
  Behren's Cellulose Ammonium Nitrate Solution ... 199
C_2. Organic carbon not supplied exclusively as carbohydrates.
  Raulin's Solution (Smith) ... 200
  Behrens' Tartrate Ammonium Nitrate Solution ... 201
  Behrens' Citrate Ammonium Nitrate Solution ... 202
  von Tubeuf's Cellulose Ammonium Nitrate Solution ... 203
B_3. Ammonia supplied as ammonium sulphate.

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*See next page for A_2.

†See B_3, B_4, B_5, B_6 and B_7.

C_1. Only one type of organic carbon added.
D_1. Organic carbon supplied as carbohydrate.
E_1. Monosaccharides added.
Charpentier's Glucose ammonium Sulphate Solution.................. 204
Artari's Cane Syrup Ammonium Sulphate Solution (Owen)............ 205
E_2. Disaccharides added.
Bokorny's Sucrose Ammonium Sulphate Solution (acid).............. 206
Mortensen's Sucrose Ammonium Sulphate Solution..................... 207
Woltje's Sucrose Ammonium Sulphate Solution (Zikes).................. 208
D_2. Organic carbon supplied as alcohols.
Bokorny's Ethyl Alcohol Ammonium Sulphate Solution.................. 209
Bokorny's Glycerol Ammonium Sulphate Solution..................... 210
D_3. Organic carbon supplied only as acids or their salts.
Behrens' Basal Malic Acid Ammonium Phosphate Solution.......... 211
C_2. More than one type of organic carbon added.
D_1. Containing carbohydrates and organic acids.
Dombrowski's Glucose Ammonium Sulphate Solution.................. 212
Bokorny's Tartrate Ammonium Sulphate Solution.................... 213
Raulin's Solution (Lode)........... 214
D_2. Containing alcohols and organic acids.
Proskauer and Beck's Basal Citrate Ammonium Sulphate Solution. (Mendel).......................... 215
B_4. Ammonia supplied as ammonium salts of phosphoric acid.
C_1. Organic carbon present as carbohydrates (other types of organic carbon may also be present).
Pasteur's Glucose Ammonium Phosphate Solution (Simanowsky)..... 216
Omeliansky's Glucose Ammonium Phosphate Solution................ 217
Henneberg's Sucrose Ammonium Acid Phosphate Solution.......... 218
Kossowicz's Sucrose Ammonium Phosphate Solution (Will)......... 219
Schukow's Glucose Ammonium Phosphate Solution.................... 220
C_2. Organic carbon supplied as fat.
Rahn's Fat-Ammonium Phosphate Solution.......................... 221
B_5. Ammonia present as ammonium salts of organic acids.
C_1. No other types of organic carbon added.
Bokorny's Ammonium Tartrate Solution.............................. 222
C_2. Other types of organic carbon added.
Pasteur's Sucrose Ammonium Tartrate Solution (Pringsheim)....... 224
Bokorny's Sucrose Ammonium Tartrate Solution..................... 225
Pringsheim's Sucrose Ammonium Tartrate Solution................. 226
A_2. Media Primarily for growth of bacteria.
B_1. Ammonia supplied as ammonium hydroxide.
Hulton-Frankel, Barber and Pyle's Acetic Acid and Ammonia Solution.............................. 227
B_2. Ammonia supplied as ammonium chloride.
C_1. Only one type of organic carbon supplied.
D_1.* Organic carbon supplied as carbohydrate.
Wherry's Levulose Ammonium Chloride Solution..................... 228
Schardinger's Sucrose Ammonium Chloride Solution................. 229
Klecki's Lactose Ammonium Phosphate Solution.................... 230
Krainsky's Starch Ammonium Chloride Solution...................... 231
Vierling's Cellulose Ammonium Chloride Solution...................... 232
D_2. Organic carbon supplied as alcohols.
Proskauer and Beck's Basal Glycerol Ammonium Chloride Solution..... 233
Higgins's Glycerol Ammonium Chloride Solution...................... 234
Revis' Glycerol Ammonium Chloride Solution......................... 235
Proskauer and Beck's Mannitol Ammonium Chloride Solution.......... 236
D_3. Organic carbon supplied as salts of organic acids.
van Delden's Lactate Ammonium Chloride Solution................. 237
Sohngen's Basal Ammonium Chloride Salt Solution.................... 238
Wherry's Ammonium Acetate Solution.............................. 239
D_4. Organic carbon supplied as hydrocarbons.

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*See D_2, D_3 and D_4.

Sohngen's Hydrocarbon Ammonium Chloride Solution.................. 240

C_2. More than one type of organic carbon supplied. (Containing alcohols together with carbohydrates or organic acids.)

Proskauer and Beck's Glucose Ammonium Chloride Solution.......... 242

Conn's Glycerol Ammonium Chloride Solution.......................... 243

Proskauer and Beck's Mannitol ammonium Chloride Solution.......... 244

B_3. Ammonia supplied as ammonium nitrate.

Mayer's Ammonium Nitrate Solution (Tanner).......................... 244a

Capaldi and Proskauer's Mannitol Ammonium Nitrate Solution....... 245

Proskauer and Beck's Glycerol Ammonium Nitrate Solution.......... 246

B_4 Ammonia supplied as ammonium carbonate.

Heraeus' Glucose Ammonium Carbonate Solution.................... 247

Lowenstein's Glycerol Ammonium Carbonate Solution................ 248

Proskauer and Beck's Basal Glycerol Ammonium Carbonate Solution... 249

B_5. Ammonia supplied as ammonium sulphate.

C_1. Only one type of organic carbon supplied.

D_1. Organic carbon supplied only as carbohydrates.

E_1. Monosaccharides only added.

Bijerinck's Glucose Ammonium Sulphate Solution.................... 250

Doryland's Glucose Ammonium Sulphate Solution.................... 251

Winogradsky's Glucose Ammonium Sulphate Solution................. 252

Gage's Glucose Ammonium Sulphate Solution.......................... 253

Stoklasa's Glucose Ammonium Sulphate Solution.................... 254

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191. Wilder's Sugar Salt Solution (Chrzaszcz)

Constituents:

1. Water	1000.0 cc.
2. Sugar	100.0 g.
3. $MgSO_4$	2.5 g.
4. KCl	2.5 g.
5. NH_4Cl	2.5 g.
6. Na_2HPO_4	2.5 g.
7. $CaCO_3$	0.5 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) A white precipitate of Ca_3PO_4 forms when $CaCO_3$ is added. This may or may not be filtered out.

(3) Distribute in flasks or tubes.

Sterilization: Not specified.

Use: To study growth requirements of yeasts. The filtered medium showed less growth of yeast than the non-filtered material.

Reference: Chrzaszcz (1904 p. 145).

192. LaGarde's Sucrose Ammonium Chloride Solution

Constituents:

1. Water	1000.0 cc.
2. Cane sugar	30.0 g.
3. NH_4Cl	6.0 g.
4. $MgSO_4$	0.5 g.
5. KH_2PO_4	0.5 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of *Phycomyces nitens, Mucor Mucedo, Mucor Rouxii, Mucor corymbifer, Mucor spinosus, Mucor racemosus, Mucor rhizopodiformis, Mucor stolonifer.*

Reference: LaGarde (1911–12 p. 248).

193. Fulmer and Nelson's Sucrose Ammonium Chloride Solutions

Constituents:

1. Water	1000.0 cc.
2. NH_4Cl	1.88 g.
3. Sucrose	100.0 g.
4. K_2HPO_4	1.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Adjustment of reaction not given.

Sterilization: Method not specified.

Use: Continuous cultivation of *Saccharomyces cerevisiae.* Authors found that these solutions gave continuous growth.

Variants:

(a) Authors added 1.0 g. $CaCl_2$ to above solution.

(b) Authors added 1.0 g. $CaCl_2$ and 6.0 g. dextrin to the above solution.

Reference: Fulmer and Nelson (1923 p. 132).

194. Mayer's Sucrose Ammonium Nitrate Solution (Pringsheim)

Constituents:

1. Water	1000.0 cc.
2. Sugar	100.0 g.
3. KH_2PO_4	5.0 g.
4. $MgSO_4$	5.0 g.
5. $Ca_3(PO_4)_2$	0.5 g.
6. NH_4NO_3	7.5 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study bios requirements of yeast. Growth and fermentation occurred after one day.

Reference: Pringsheim (1906 p. 114).

195. Bokorny's Sucrose Ammonium Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. NH_4NO_3 (0.47%)	4.7 g.
3. $(NH_4)_2SO_4$ (0.005%)	0.05 g.
4. Cane sugar (9.0%)	90.0 g.
5. Dipotassium phosphate (0.035%)	0.35 g.
6. $MgSO_4$ (0.036%)	0.36 g.
7. $CaCl_2$ (0.0015%)	0.015 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: To study yeast growth.
Reference: Bokorny (1911 p. 182).

196. Hollborn's Sucrose Ammonium Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. Sugar	50.0 g.
3. $(NH_4)NO_3$	10.0 g.
4. KH_2PO_4	5.0 g.
5. $MgSO_4$	2.5 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of *Tricophyton rodens* causing "Alopecia areata," one kind of baldness. Growth similar to that in bouillon. Growth on the surface—first olive green and later darker green. Similar medium used to study fermentation by *Aspergillus niger*.

Variants: Currie recommended the use of the following medium to study citric acid fermentation of *Aspergillus niger*:

1. Water	1000.0 cc.
2. Sucrose	125.0–150.0 g.
3. NH_4NO_3	2.0–2.5 g.
4. KH_2PO_4	0.75–1.0 g.
5. $MgSO_4 \cdot 7H_2O$	0.2–0.25 g.

6. HCl to give pH between 3.4–3.5 (4–5 cc. N/5 HCl).

197. Laborde's Sucrose Ammonium Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. Sugar	50.0 g.
3. NH_4NO_3	2.0 g.
4. Potassium phosphate	1.0 g.
5. $MgSO_4$	0.1 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of the mold *Enrotiopsis Gayohi*.

Reference: Laborde (1897 p. 13).

198. Ono's Sucrose Ammonium Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. Cane sugar	85.0 g.
3. NH_4NO_3	16.0 g.
4. $MgSO_4$	3.0 g.
5. KCl	3.0 g.
6. $NH_4H_2PO_4$	3.0 g.
7. $FeSO_4$	trace
8. $CuSO_4$ solution	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Distribute in 30.0 cc. lots.
(3) Add $CuSO_4$ so that the flasks will contain from 1/250 to 1/32,000 Mol $CuSO_4$.

Sterilization: Sterilize in the steamer.

Use: To study the effect of small amounts of $CuSO_4$ on the growth of *Aspergillus niger*. It was found that small amounts of $CuSO_4$ tend to increase growth. Higher concentration than 1/250 tend to hinder growth.

Reference: Ono (1902 p. 155).

199. Behrens' Cellulose Ammonium Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. NH_4NO_3	10.0 g.
3. Potassium phosphate	5.0 g.
4. $MgSO_4$	2.5 g.
5. Filter paper (Swedish)	50.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) After sterilization add 5.0 g. of purified Swedish filter paper containing some of the mycelium.

Sterilization: Method not given.

Use: Cultivation of *Pseudo-Dematophora*. Growth was luxuriant. Utilized cellulose, as a source of carbon.

Reference: Behrens, (1897 p. 640).

200. Raulins Solution (Smith)

Constituents:

1. Water	1500.0 cc.
2. Sugar	70.0 g.
3. Tartaric acid	4.0 g.
4. NH_4NO_3	4.0 g.
5. Ammonium Phosphate	0.6 g.
6. K_2CO_3	0.6 g.
7. $MgSO_4$	0.4 g.
8. $(NH_4)_3SO_4$	0.25 g.
9. $ZnSO_4$	0.07 g.
10. $FeSO_4$	0.07 g.
11. K_2SiO_3	0.07 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 in 1.

Sterilization: Not specified.

Use: Cultivation of *Aspergillus niger* and other molds.

Variants:

(a) Bezançon did not specify the type of ammonium phosphate, and specified that iron sulphate was to be used.

(b) Harvey omitted the $MgCO_3$ and did not specify the type of ammonium phosphate.

(c) Dopter and Sacquépée, and Heinemann, did not specify the type of ammonium phosphate.

(d) Tanner omitted the K_2CO_3.

(e) Harvey also gave the following solution:

1. Distilled water	1500.0 cc.
2. NH_4NO_3	4.5 g.
3. Sucrose	70.0 g.
4. Potassium tartrate	6.5 g.
5. K_2HPO_4	0.6 g.
6. $MgCO_3$	0.4 g.
7. K_2SO_4	0.25 g.
8. $FeSO_4$	0.07 g.
9. $ZnSO_4$	0.07 g.
10. K_2SiO_3	0.07 g.

(f) Betrand and Javillier gave the following modification:

1. Water	1000.0 cc.
2. Sugar	46.6 g.
3. Tartaric acid	2.66 g.
4. K_2CO_3	0.4 g.
5. NH_4NO_3	2.66 g.
6. Ammonium phosphate	0.4 g.
7. $(NH_4)_2SO_4$	0.155 g.
8. $MgSO_4$	0.71 g.
9. Iron aluminum	0.081 g.
10. K_2SiO_3	0.046 g.

(g) Roux and Rochaix did not specify the type of ammonium phosphate used, used 0.4 g. $MgCO_3$ instead of $MgSO_4$, and 0.07 g. K_2SO_4 instead of K_2SiO_3.

References: Smith (1905 p. 197), Roux and Rochaix (1911 p. 104), Brenner (1914 p. 556), Bezançon (1920 p. 20), Besson (1920 p. 36) Harvey (1921–22 p. 103), Dopter and Sacquépée (1921 p. 16), Tanner (1919 p. 66), Heinemann (1922 p. 42), Betrand and Javillier (1912 p. 244).

201. Behrens' Tartrate Ammonium Nitrate Solution.

Constituents:

1. Water	1000.0 cc.
2. Sucrose	50.0 g.
3. Tartaric acid	5.0 g.
4. NH_4NO_3	5.0 g.
5. K_2HPO_4	2.0 g.
6. $MgSO_4$	1.0 g.

7. Tannin 0.0, 1:1000, 1:500, 1:100 or 1:50.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Distribute in 50.0 cc. lots.

Sterilization: Method not given.

Use: Cultivation of Botrytis. Tannin inhibits growth, 0.5 and 1.0 g. per flask showed no growth of the spores.

Variants: The author added 0.0, 0.05 g. (1:1000), 0.1 g. (1:500), 0.5 g. (1:100) or 1.0 g. (1:50) of tannin to each flask before sterilization.

Reference: Behrens' (1898 p. 642).

202. Behrens' Citrate Ammonium Nitrate Solution.

Constituents:

1. Water	1000.0 cc.
2. Cane sugar	100.0 g.
3. NH_4NO_3	20.0 g.
4. Citric Acid	10.0 g.
5. Potassium phosphate	10.0 g.
6. $MgSO_4$	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1 liter of water rich in potassium.

(2) Distribute in 50.0 cc. lots in 300.0 cc. flasks (28 flasks).

Sterilization: Method not given.

Use: To determine influence of chemicals on growth of *Pseudodematephaca*. Growth takes place in copper sulphate 1:100,000, 1:10,000 and 1:5000. In iron sulphate 1:100,000 to 1:500; in sodium fluoride 1:100,000, and in potassium sulfocarbonate 1:100,000 to 1:500.

Added materials: The author added the following materials:

(a) 1.0 and 0.1% solutions of copper vitrol, iron vitrol or sodium fluoride so that the concentration would be 1:100, 1:200, 1:500, 1:1000, 1:5000, 1:10,000, 1:100,000 (previous to sterilization).

(b) 1.0% and 0.1% sterile solutions of potassium sulphocarbonate so that the concentration would be 1:100, 1:200, 1:500, 1:1000, 1:5000, 1:10,000, 1:100,000 (added to the sterilized medium under aseptic conditions).

Reference: Behrens' (1897 p. 745).

203. von Tubeuf's Cellulose Ammonium Nitrate Solution.

Constituents:

1. Water	1000.0 cc.
2. NH_4NO_3	10.0 g.
3. Potassium phosphate	5.0 g.
4. $MgSO_4$	1.0 g.
5. Lactic acid	2.0 g.
6. Cellulose	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Distribute in 50.0 cc. lots.

(3) Prepare a series of flasks by adding 10.0 g. filter paper (Swedish) or 10.0 g. ordinary filter paper, or 10.0 g. of pine shavings or 10.0 g. of wadding (10.0 g. sucrose with 5.0 g. gelatin were also added).

Sterilization: Not specified.

Use: Cultivation of *Merulius lacrymans* (causing dry rot). Good growth using Swedish filter paper as carbon source. Little growth on shavings or wadding. Good growth on ordinary malt and meat extract containing cane sugar and citric acid 1 to 3%.

Reference: v Tubeuf (1902 p. 130).

204. Charpentier's Glucose Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose	10.0 g.
3. $FeSO_4$	trace
4. $(NH_4)_2SO_4$	0.5 g.
5. $CaCl_2$	0.023 g.
6. K_2HPO_4	2.0 g.
7. $MgSO_4$	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Distribute in 100.0 cc. lots.

Sterilization: Not specified.

Use: Nitrogen assimilation by Algae, *Cystococcus humicola.*

Reference: Charpentier (1903 p. 330).

205. Artari's Syrup Ammonium Sulphate Solution (Owen)

Constituents:

1. Water	1000.0 cc.
2. Na_2HPO_4 (0.15%)	0.15 g.
3. $MgSO_4$ (0.1%)	0.1 g.
4. $(NH_4)_2SO_4$ (0.15%)	0.15 g.
5. Grape sugar (2.0%)	2.0 g.
6. Cane syrup	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1. This is Artari's solution.

(2) Prepare the following modifications with cane syrup. Preparation of syrup or exact composition not given (may be 50.0% cane sugar solution).

  (a) Make no additions to cane syrup.

  (b) To 2 parts cane syrup, add 1 part (1).

  (c) Add an equal amount of (1) to cane syrup.

  (d) To 1 part cane syrup add 2 parts (1).

(3) Tube in about 10.0 cc. lots.

Sterilization: Steam for 15 minutes on each of 3 successive days.

Use: To study effect of density on growth of *Saccharomyces Zopfii.* Cells are smaller in solutions of greatest density. Yeast grown in thick syrups seem to retain their fermentation ability when subjected to high temperatures, more than yeast grown in a medium of lesser density.

Reference: Owen (1913 p. 473).

206. Bokorny's Sucrose Ammonium Sulphate Solution (acid)

Constituents:

1. Distilled water	1000.0 cc.
2. Cane Sugar	50.0 g.
3. KH_2PO_4	1.0 g.
4. $MgSO_4$	1.0 g.
5. $(NH_4)_2SO_4$	5.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Growth of yeast.

Reference: Bokorny (1902 p. 56), (1917 p. 369).

207. Mortensen's Sucrose Ammonium Sulphate Solution

Constituents:

1. Water	100.0 cc.
2. Cane sugar (10.0%)	10.0 g.
3. $(NH_4)_2SO_4$ (0.5%)	0.5 g.
4. Cobalt salt	

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add cobalt salts in varying amounts from 4 to 1/32%.

Sterilization: Not specified.

Use: To study the effect of cobalt salts on the growth of *Aspergillus niger.* The author found that the toxic properties of cobalt are different in different media.

Reference: Mortensen (1909 p. 532).

208. Wöltje's Sucrose Ammonium Sulphate Solution (Zikes)

Constituents:

1. Water	100.0 cc.
2. $(NH_4)_2SO_4$	1.0 g.
3. $MgSO_4$	0.25 g.
4. Saccharose	7.5 g.
5. K_2HPO_4	0.5 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not given.

Use: To study perithecium formation by *Aspergillus oryzae*.

Reference: Zikes (1922 p. 340).

209. Bokorny's Ethyl Alcohol Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. Ethyl alcohol (0.2%)	20.0 g.
3. $(NH_4)_2SO_4$ (0.02%)	0.2 g.
4. K_2HPO_4 or KH_2PO_4 (0.05%)	0.5 g.
5. $MgSO_4$	0.2 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not given.

Use: Growth of fungi, *Schizomycetes*, molds and yeast.

Reference: Bokorny (1917 p. 195).

210. Bokorny's Glycerol Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerin	5.0 g.
3. KH_2PO_4	0.1 g.
4. Bittersalts $MgSO_4$	0.1 g.
5. $(NH_4)_2SO_4$	0.5 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not given.

Use: Cultivation of yeast.

Reference: Bokorny (1911 p. 182).

211. Behrens' Basal Malic Acid Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2SO_4$	20.9 g.
3. Malic acid	90.0 g.
4. $MgSO_4$	5.0 g.
5. Potassium phosphate	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Distribute in 10.0 cc. lots.

(3) Add 1.0 g. of one of the added nutrients to each tube.

Sterilization: Usual method. (Method not given.)

Use: Cultivation of *Penicillium glaucum* and *Botrytis vulgaris*. Growth was more luxuriant in medium containing pectin than in arabinose medium.

Added nutrients: The author added 1.0 g. of arabinose or calcium pectate (from flax) to each 10.0 cc. lot.

Variants: The author made no additions to the basic solution.

Reference: Behrens (1898 p. 552).

212. Dombrowski's Glucose Ammonium Sulphate Solution.

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $MgSO_4$	0.5 g.
4. Dextrose	100.0 g.
5. Lactic acid	
6. $(NH_4)_2SO_4$	0.5 to 5.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Acidify slightly with lactic acid

(3) Add 0.05, 0.1, 1.0, 2.0 or 5.0 g. of $(NH_4)_2SO_4$ to 100.0 cc. of (2).

Sterilization: Not specified.

Use: To study nitrogen requirements for milk yeasts, *Saccharomyces lactis* and *Zygosaccharomyces lactis*. Peptone was found to be the best nitrogen source.

Variants: The author substituted 20.0 to 50.0 g. of asparagin or 2.0 to 50.0 g. of Witte's peptone for the $(NH_4)_2SO_4$.

Reference: Dombrowski (1910 p. 393).

213. Bokorny's Tartrate Ammonium Sulphate Solution

Constituents:

1. Water	1000.0	cc.
2. Cane sugar (10.0%)	100.0	g
3. Ammonium tartrate (0.5%)	5.0	g.
4. K_2HPO_4 (0.035%)	0.35	g.
5. $MgSO_4$ (0.006%)	0.06	g.
6. $CaCl_2$ (0.0015%)	0.015	
7. $(NH_4)_2SO_4$ (0.0061%)	0.061	g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not given.

Use: Cultivation of yeast. The culture may or may not be treated with a current of air.

Reference: Bokorny (1917 p. 368).

214. Raulin's Solution (Lode)

Constituents:

1. Water	1500.0 cc.
2. Sugar candy	70.0 g.
3. Tartaric acid	4.0 g.
4. Ammonium phosphate	0.6 g.
5. $CaCO_3$	0.6 g.
6. $MgCO_3$	0.4 g.
7. $(NH_4)_2SO_4$	0.25 g.
8. $FeSO_4$	0.07 g.
9. $ZnSO_4$	0.07 g.
10. Calcium silicate	0.07 g.
11. Ammonium acetate	4.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 in 1.

Sterilization: Not specified.

Use: Cultivation of molds, *Aspergillus.*

Reference: Lode (1902 p. 125).

215. Proskauer and Beck's Basal Citrate Ammonium Sulphate Solution (Mendel)

Constituents:

1. Distilled water	1000.0 cc.
2. $(NH_4)_2SO_4$	3.0 g.
3. Citric acid	2.0 g.
4. $MgSO_4$	2.0 g.
5. Potassium biphosphate	5.0 g.
6. Glycerin	15.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Adjustment of reaction not specified.
(3) Add 1.0% of one of the carbon sources listed under added nutrients.

Sterilization: Sterilize for 30 minutes on each of two successive days. On the third day add 1.0% of glucose and sterilize for 15 minutes. (Method of sterilization not specified.)

Use: Cultivation of *Bacterium coli* and *Bacterium Fitzianus.*

Added nutrients: The author used 1.0% of the following carbohydrates as carbon sources:

glucose
lactose
maltose
sucrose

Reference: Mendel (1911 p. 296).

216. Pasteur's Glucose Solution (Simanowsky)

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4	5.0 g.
3. $(NH_4)H_2PO_4$	5.0 g.
4. Glucose	80.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not given.

Use: Cultivation of yeast.

Variants: The author substituted 80.0 g. of sucrose for glucose.

Reference: Simanowsky (1886 p. 22).

217. Omeliansky's Glucose Ammonium Phosphate Solution

Constituents

1. Water	1000.0 cc.
2. Glucose	100.0 g.
3. Ammonium phosphate	2.0 g.
4. KCl	0.1 g.
5. $MgSO_4$	0.1 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Kind of phosphate salt not specified.
(3) Adjustment of reaction not specified.
(4) Place solution in thin layer in Winogradsky flask.

Sterilization: Method not given.

Use: Cultivation of aroma producing yeast and the preservation of stock cultures. Gives table wine aroma. Cane sugar cannot replace glucose.

Reference: Omeliansky (1923 p. 418).

218. Henneberg's Sucrose Ammonium Acid Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2HPO_4$ (0.2%)	2.0 g.
3. KH_2PO_4 (0.2%)	2.0 g.
4. $MgSO_4$ (0.1%)	1.0 g.
5. Sucrose (15.0%)	150.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of yeast.

Variants: The author suggested the addition of one of the following:

Gypsum (2.0%)	20.0 g.
Potassium phosphate (2.0%)	20.0 g.
$CaCl_2$ (0.6%)	6.0 g.
Potassium lactate (2.0%)	20.0 g.

K_2CO_3 (2.0%)	20.0 g.
Soda (0.6%)	6.0 g.
K_2SO_4 (0.6%)	6.0 g.

Reference: Henneberg (1909 p. 105).

219. Kossowicz's Sucrose Ammonium Phosphate Solution (Will)

Constituents:

1. Water	1000.0 cc.
2. Saccharose (5.0%)	50.0 g.
3. KCl (0.4%)	4.0 g.
4. $(NH_4)_2HPO_4$ (0.4%)	4.0 g.
5. $MgSO_4$ (0.4%)	4.0 g.
6. $CaHPO_4$ (0.04%)	0.4 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of non-spore forming *Saccharomyces*.

Reference: Will (1908 p. 387).

220. Schukow's Glucose Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. Ammonium phosphate	5.0 g.
3. Potassium phosphate	1.0 g.
4. $MgSO_4$	0.5 g.
5. Glucose	10.0 g.
6. Organic acid	90.0 g. to 100.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add 9 to 10.0% of an organic acid to (1).

(3) Adjustment of reaction not specified.

(4) Distribute into fermentation flasks (sealed with H_2SO_4).

(5) After sterilization and inoculation plug the flasks with a cork and sear with paraffin.

Sterilization: Sterilize in the steamer (method not given).

Use: To study utilization of acids by yeast. After 75 days 0.11 g. tartaric, 0.193 g. malic, 0.34 g. citric and 0.09 g. succinic acid were used. Acid determined by titration with N/10 NaOH using litmus as an indicator.

Variants: The author used one of the following organic acids:

tartaric acid
malic acid
citric acid
succinic acid

Reference: Schukow (1896 p. 607).

221. Rahn's Fat-Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	5.0 g.
3. $(NH_4)_3PO_4$	5.0 g.
4. $MgSO_4$	1.0 g.
5. $CaCl_2$	1.0 g.
6. $FeCl_3$	trace
7. HCl	trace
8. Fat	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Melt fat and pour in an Erlenmeyer flask and incline the flask in such a position so that when it solidifies only a portion of the fat will be immersed in the solution.

(3) Neutralize (1) with 10.0% NaOH using litmus as an indicator. Do not filter.

(4) Add (3) to the flasks containing fat, solidified on the wall of the flask. Do not add enough (3) to immerse all the fat.

Sterilization: Not specified.

Use: To study fat decomposition by *Penicillium glaucum*. Fat was decomposed.

Reference: Rahn (1905 p. 423).

222. Bokorny's Ammonium Tartrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium tartrate	2.0 g.
3. KH_2PO_4	1.0 g.
4. $MgSO_4$	0.5 g.
5. Iron chloride	trace
6. $CaSO_4$	0.0 or 1.0 g.

Preparation: Dissolve 2, 3, 4, 5 and 6 in water that has been distilled in quartz containers.

Sterilization: Not specified.

Use: To study effect of metallic salts on yeast. No growth took place in the solution lacking calcium.

Reference: Bokorny (1912 p. 135).

224. Pasteur's Sucrose Ammonium Solution (Pringsheim)

Constituents:

1. Water	1000.0 cc.
2. Sucrose	100.0 g.
3. Ammonium tartrate	10.0 g.
4. KH_2PO_4	5.0 g.
5. $Ca_3(PO_4)_2$	1.0 g.
6. $Mg_3(PO_4)_2$	1.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study bios requirements of yeast. Fermentation took place after one day.

Reference: Pringsheim (1906 p. 114).

225. Bokorny's Sucrose Ammonium Tartrate Solution

Constituents:

1. Water	1000.0 cc.
2. Saccharose (Kahlbaum 10.0%)	100.0 g.
3. NaK_2PO_4 (0.1%)	1.0 g.
4. Ammonium tartrate (0.25%)	2.5 g.
5. $MgSO_4$ (0.05%)	0.5 g.
6. KCl	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add 0.0%, 0.025%, 0.05%, 0.1%, 0.25%, 0.5% or 1.0% KCl to 1.

Sterilization: Not specified.

Use: To study effect of metalic salts on yeast. Potassium reported necessary for the growth of the yeast.

Reference: Bokorny (1912 p. 123).

226. Pringsheim's Sucrose Ammonium Tartrate Solution

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4	2.0 g.
3. $MgSO_4$	0.4 g.
4. $CaCl_2$	0.2 g.
5. Ammonium tartrate	10.0 g.
6. Sucrose	100.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study bios requirements of yeast. The author reported fermentation after one day in this medium.

Reference: Pringsheim (1906 p. 114).

227. Hulton-Frankel, Barber and Pyle's Acetic Acid and Ammonia Solution

Constituents:

1. Water	1000.0 cc.
2. H_3PO_4 (12.79 g) M soln	129.5 cc.
3. CH_3COOH (1.13 g) M soln	18.8 cc.
4. NH_4OH (0.62 g) M soln	17.8 cc.
5. 0.01% $CaCl_2$ (0.001 g)	10.0 cc.
6. 0.01% $FeCl_3$ (0.001 g)	10.0 cc.
7. 0.01% $MgSO_4$ (0.001 g)	10.0 cc.
8. NaOH (4.0 g) M soln	100.0 cc.
9. KOH (5.6 g) M soln	100.0 cc.

Preparation:

(1) Dilute solutions 23, and 4 to 500.0 cc. with 1.

(2) Add to (1), 5, 6 and 7 and equal volumes of M solution of 8 and 9 to give pH of 7.0.

(3) Solution is water clear.

(4) Tube into sterile tubes.

(5) pH of medium may be adjusted by varying amounts of alkali added. Medium may be prepared using molar solutions or by weight.

Sterilization: Steam on each of 3 successive days for 15 minutes.

Use: General culture medium.

Variants: Hulton-Frankel and Barber used the same solution as a basic medium with litmus or Andrade's indicator to which they added 10.0 g. of one of the following:

(a) sucrose	(c) lactose
(b) glucose	(d) mannitol

References: Hulton-Frankel-Barber and Pyle (1917 p. 17), Hulton-Frankel and Barber (1917 p. 17), Harvey (1921–22 p. 104).

228. Wherry's Levulose Ammonium Chloride Solution.

Constituents:

1. Redistilled water	1000.0 cc.
2. NH_4Cl	2.0 g.
3. Levulose	2.0 g.
4. Calcium phosphate (primary, secondary or tertiary)	2.0 g.

Preparation:

(1) Dissolve 2, 3 and one of 4 in 1.

(2) Adjustment of reaction not given.

Sterilization: Autoclave (time not specified).

Use: To study acid proofness of *B. tuberculosis*. Primary salt—no growth; secondary salt—growth good—non-acid proof rods, some "free spores." Tertiary salt—growth good—smaller non-acid proof rods, many "free spores."

Variants: The author gave the following variants:

(a) Used the same solution but substituted primary, secondary, tertiary meta or pyro sodium phosphate for calcium phosphate. These solutions gave the following results:

Primary salt growth good at 48 hours—part are strongly acid proof, some "spores."

Secondary salt growth good at 48 hours—part are strongly acid proof, some "spores."

Tertiary salt growth good. Non-acid proof.

Meta no growth.

Pyrophosphate growth good. Rods are chiefly short and non-acid proof. Some are larger and longer and are strongly acid proof.

(b) Substituted 2.0 g. K_2HPO_4 for calcium phosphate. Bacilli non-acid proof.

(c) Substituted 2.0 g. KH_2PO_4 for calcium phosphate. Acid proof rods were often club shaped.

Reference: Wherry (1913 p. 150).

229. Schardinger's Sucrose Ammonium Chloride Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Sucrose	50.0 to 80.0 g.
3. NH_4Cl	5.0 g.
4. KH_2PO_4	1.0 g.
5. $MgSO_4$	0.5 g.
6. $CaCO_3$	10.0 to 15.0 g.

Preparation: (1) Dissolve 2, one of 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Study of slime production. Fermentation starts after 24 hours. The slime forms at the bottom of the flask.

Variants: The author suggested the use of $(NH_4)_2SO_4$ instead of NH_4Cl.

Reference: Schardinger (1902 p. 177).

230. Klecki's Lactose Ammonium Phosphate Solution

Constituents:

1. Water	1000.0	cc.
2. Lactose	20.0	g.
3. $CaCO_3$	3.0	g.
4. NH_4Cl	2.0	g.
5. Potassium phosphate	0.058	g.
6. Ammonium phosphate	0.1053	g.
7. $MgSO_4$	0.055	g.
8. $(NH_4)_2SO_4$	0.023	g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

(2) Adjustment of reaction not given.

(3) Distribute in Pasteur fermentation tubes.

Sterilization: Method not given.

Use: Cultivation of *Bacillus saccharobutyricus*. Fermentation takes place after about 60 hours after inoculation.

Reference: Klecki (1896 p. 255).

231. Krainsky's Starch Ammonium Chloride Solution.

Constituents:

1. Water	1000.0 cc.
2. Starch (1.0%)	10.0 g.
3. NH_4Cl (0.05%)	0.5 g.
4. K_2HPO_4 (0.05%)	0.5 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Isolation and enrichment of *Actinomyces*. After 2 days because of bacterial increase, a weak coloration and a slight H_2S odor appears. The actinomycetes colonies swim on the surface. Inoculate these colonies in a new medium of the same composition and a heavy scum is formed.

Variants: The author substituted 2 to 3.0% cellulose for 1.0% starch.

Reference: Krainsky (1914 p. 657).

232. Vierling's Cellulose Ammonium Chloride Solution

Constituents:

1. Water	1000.0 cc.
2. $CaCO_3$ (0.1%)	1.0 g.
3. (NH_4) Cl (0.1%)	1.0 g.
4. Filter paper.	

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Place strips of filter paper in flasks.

(3) Pour (1) into each flask containing filter paper.

(4) Seal the flasks with paraffin after inoculation.

Sterilization: Not specified.

Use: Decomposition of cellulose by *Myco-*

bacteria. Growth occurs. Filter paper shows no signs of being attacked.

Reference: Vierling (1920 p. 206).

233. Proskauer and Beck's Basal Glycerol Ammonium Chloride Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol (1.5%)	15.0 g.
3. KH_2PO_4 (0.5%)	5.0 g.
4. $MgSO_4$ (0.25%)	2.5 g.
5. NH_4Cl (0.2%)	2.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add one of the listed carbon sources.

Sterilization not specified.

Use: To study requirements for growth for the tubercle bacilli.

Added nutrients: The author added the following carbohydrates and alcohols as carbon sources:

maltose 1.0%
lactose 1.0%
sucrose 1.0%
raffinose 1.0%
mannitol 0.6%
dulcitol 0.6%
isodulcitol 0.6%

Reference: Proskauer and Beck (1894 p. 146).

234. Higgins' Glycerol Ammonium Chloride Solution

Constituents:

1. Water	100.0 cc.
2. Glycerin	5.0 g.
3. NH_4Cl	0.8 g.
4. Potassium phosphate	0.15 g.
5. Potassium sulphate	0 or 0.1 g.

Preparation:

(1) Dissolve 2, 3 and 4 and 5 in 1.

(2) Neutralize to phenolphthalein.

Sterilization: Method not given.

Use: Cultivation of cholera organisms.

Reference: Higgins (1898 p. 666).

235. Revis' Glycerol Ammonium Chloride Solution

Constituents:

1. Water	1000.0 cc.
2. NH_4Cl	10.0 g.
3. Glycerol	10.0 g.
4. Potassium phosphate	1.0 g.
5. $MgSO_4$	0.2 g.
6. $CaCl_2$	0.1 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study stability of physiological properties of colon and coli form organisms. No distinctive changes were produced in this non-albuminous medium, but the vigor of fermentation varied somewhat. The organisms show a greater tendency to die off in this medium than in ordinary media.

Reference: Revis (1910 p. 173).

236. Proskauer and Beck's Mannitol Ammonium Chloride Solution

Constituents:

1. Water	1000.0 cc.
2. Mannitol (0.6%)	6.0 g.
3. NH_4Cl (0.2%)	2.0 g.
4. KH_2PO_4 (0.5%)	5.0 g.
5. $MgSO_4$ (0.25%)	2.5 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: To study growth requirements of the tubercle bacillus. The authors reported no growth unless glycerol was present.

Reference: Proskauer and Beck (1894 p. 147).

237. van Delden's Lactate Ammonium Chloride Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl	30.0 g.
3. K_2HPO_4	0.05 g.
4. NH_4Cl	1.0 g.
5. Sodium lactate	10.0 g.
6. $MgSO_4 \cdot 7H_2O$	(amount not specified)

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study sulphate reduction by *M. aestuarii*.

Variants: The author used 0.25 g. K_2HPO_4, 0.25 g. NH_4Cl, specified the use 2.0 g. $MgSO_4 \cdot 7H_2O$ and omitted the NaCl.

Reference: van Delden (1903–4 pp. 117–118).

238. Söhngen's Basal Ammonium Chloride Salt Solution.

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	0.2 g.
3. NH_4Cl	0.2 g.
4. Seignettes salt (sodium potassium tartrate)	0.2 g.

Preparation:
(1) Dissolve 2, 3, and 4 in 1000.0 cc. of water.
(2) Add one of the materials listed under added nutrients.
(3) Adjustment of reaction not given.
(4) Sterilize the solution and colloids separately.
(5) Incubate 24 hours to test sterility.

Sterilization: Method not specified.

Use: To study influence of colloids on bacterial process of *B. fluorescens liquefaciens*. After two days cultures in (2) (a), (c), (e), (h) are turbid white, (d), (f), (g) are clear. Growth obtained in all cases however.

Added nutrients: The author added one of the following combinations:
(a) 5.0 g. distilled water
(b) 5.0 g. distilled water + 0.5 g. blood charcoal
(c) 5.0 g. distilled water + 0.5 g. blood charcoal + 0.2 g. $CaCO_3$
(d) 5.0 g. distilled water + 0.5 g. finely divided peat
(e) 5.0 g. distilled water + 0.5 g. finely divided peat + 0.2 g. $CaCO_3$
(f) 5.0 g. distilled water + 0.5 g. finely cut filter paper
(g) 5.0 cc. of a 2.0% colloidal silicum dioxide solution
(h) 5.0 cc. of a 1.0% colloidal iron oxide solution

Reference: Söhngen (1913 p. 625).

239. Wherry's Ammonium Acetate Solution.

Constituents:

1. Redistilled water	1000.0 cc.
2. Na_2CO_3 or NaCl	1.0 g.
3. KCl	1.0 g.
4. $CaCl_2$	1.0 g.
5. $MgSO_4$	1.0 g.
6. Ammonium acetate	2.0 g.
7. NH_4Cl	2.0 g.
8. KH_2PO_4	0.0 or 2.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.
(2) Adjustment of reaction not given.

Sterilization: Sterilize in autoclave, time not given.

Use: To study acid proofness of *B. tuberculosis*. Medium unfavorable.

Reference: Wherry (1913 p. 148).

240. Söhngen's Hydrocarbon Ammonium Chloride Solution.

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	0.5 g.
3. NH_4Cl	0.5 g.
4. $CaCO_3$	trace
5. Paraffin (1.0%)	10.0 g.

Preparation:
(1) Dissolve 2, 3, and 4 in 1.
(2) Heat (1) slightly and add 1.0% melted paraffin. Shake thoroughly and then cool quickly. Or add several drops of petroleum.
(3) Distribute in 100.0 cc. lots in 450.0 cc. Erlenmeyer flasks.
(4) Inoculate with garden soil.

Sterilization: Not specified.

Use: Cultivation of paraffin oxidizers. Soil forms. *B. fluorescens liquefaciens*, *B. punctatum*, *Micrococsus paraffinae*, *B. lipolyticum*.

Variants: Variants used to study the effect of a colloid upon oxidation.
(a) The author substituted several drops of petroleum for paraffin.
(b) The author substituted 20.0 cc. of petroleum for the paraffin and added 2.0 g. of colloidal silicon dioxide or colloidal iron oxide to the solution. He found that the amount of CO_2 formed was increased by the presence of a colloid, when using *Mycobacterium album*, *Mycobacterium rubrum*, *Micrococcus paraffinae*.

Reference: Söhngen (1913 p. 597), (1913 p. 644.

242. Proskauer and Beck's Glucose Ammonium Chloride Solution.

Constituents:

1. Water	1000.0 cc.
2. Glucose (1.0%)	10.0 g.
3. Citric acid (0.075%)	0.75 g.
4. NH_4Cl (0.2%)	2.0 g.
5. Glycerol (1.5%)	15.0 g.
6. KH_2PO_4 (0.5%)	5.0 g.
7. $MgSO_4$ (0.25%)	2.5 g.
8. K_2SO_4 (0.25%)	2.5 g.
9. NaCl (0.12%)	1.2 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

Sterilization: Not specified.

Use: To study growth requirements of tubercle bacilli.

Variants: The author omitted the citric acid.

Reference: Proskauer and Beck (1894 p. 142).

243. Conn's Glycerol Ammonium Chloride Solution.

Constituents:

1. Water	1000.0 cc.
2. Glycerol	10.0 cc.
3. Malic acid	4.0 g.
4. NH_4Cl	0.5 g.
5. K_2HPO_4	0.5 g.
6. $CaCl_2$	2.5 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Adjust the reaction to about neutral ($pH = 7.0$) by the addition of NaOH (about 10.0 cc. of normal solution).

Sterilization: Not specified.

Use: Cultivation of *Actinomycetes*.

Variants: The author omitted the $CaCO_3$ and used citric acid instead of malic acid.

Reference: Conn (1921 p. 7).

244. Proskauer and Beck's Mannitol Ammonium Chloride Solution.

Constituents:

1. Water	1000.0 cc.
2. Mannitol (0.6%)	6.0 g.
3. Glycerol (1.5%)	15.0 g.
4. NH_4Cl (0.2%)	2.0 g.
5. KH_2PO_4 (0.5%)	5.0 g.
6. $MgSO_4$ (0.25%)	2.5 g.
7. Magnesium citrate (0.25%)	2.5 g.
8. Citric acid (0.075%)	0.75 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: To study growth requirements of the tubercle bacillus. Authors reported no growth unless glycerol was present.

Reference: Proskauer and Beck (1894 p. 147).

244a. Mayer's Ammonium Nitrate Solution (Tanner)

Constituents:

1. Distilled water	1000.0 cc.
2. $MgSO_4 \cdot 7H_2O$	10.0 g.
3. NH_4NO_3	15.0 g.
4. $Ca_3(PO_4)_2$	0.01 g.
5. KH_2PO_4	10.0 g.
6. Sugar (kind not specified).	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Cool and add sugar (Kind and amount not specified).

Sterilization: Not specified.

Use: General culture medium.

Variants: Add 3.0% NaCl if the medium is to be used for luminous bacteria, and an excess of $CaCO_3$ if acid forming bacteria are to be grown.

Reference: Tanner (1919 p. 62).

245. Capaldi and Proskauer's Mannitol Ammonium Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. NH_4NO_3 (0.2%)	2.0 g.
3. Mannite (0.2%)	2.0 g.
4. NaCl (0.02%)	0.2 g.
5. KH_2PO_4 (0.02%)	0.2 g.
6. $MgSO_4$ (0.01%)	0.1 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Acid production by *B. coli*. Authors found that *B. coli* produced acid in this medium.

Reference: Capaldi and Proskauer (1896 p. 456).

246. Proskauer and Beck's Glycerol Ammonium Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose (1.0%)	10.0 g.
3. Glycerol (1.5%)	15.0 g.
4. KH_2PO_4 (0.5%)	5.0 g.
5. $MgSO_4$ (0.25%)	2.5 g.
6. NH_4NO_3 (0.2%)	2.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study food requirements of tubercle bacilli.

Reference: Proskauer and Beck (1894 p. 142).

247. Heraeus' Glucose Ammonium Carbonate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Potassium phosphate	0.05 g.

3. $MgSO_4$	0.01 g.
4. $CaCl_2$	0.05 g.
5. Dextrose	1.0 g.
6. $(NH_4)_2CO_3$	1.0 g.

Preparation:

(1) Dissolve 1.0 g. potassium phosphates 0.2 g. $MgSO_4$ and 0.1 g. $CaCl_2$ in 1000.0 cc. distilled water

(2) Steam for 1 hour.

(3) Allow to stand.

(4) Filter and boil again.

(5) Prepare solutions of dextrose and $(NH_4)_2CO_3$, the ratio of 1:15.

(6) Mix 10.0 cc. (4), 1.0 cc. of the dextrose solution and 1.0 cc. of $(NH_4)_2CO_2$ solution.

(7) Dilute to 200.0 cc. with distilled water. A liter of this solution contains:

0.05 g. potassium phosphate
0.01 g. MgSO
0.05 g. $CaCl_2$
1.0 g. dextrose
1.0 g. NH_4CO_3

(8) Distribute in 50.0 cc. flasks that have previously been plugged and sterilized in the sterilizer.

Sterilization: Heat the flasks in the steamer for 30 minutes.

Use: General culture medium.

Reference: Heraeus (1913 p. 224).

248. Löwenstein's Glycerol Ammonium Carbonate Solution

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2CO_4$ (Commercial)	3.5 g.
3. Potassium phosphate (primary)	1.5 g.
4. $MgSO_4$	2.5 g.
5. Glycerin	15.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

Reference: Löwenstein (1913 p. 592). Harvey (1921–22 p. 103).

249. Proskauer and Beck's Basal Glycerol Ammonium Carbonate Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol (1.5%)	15.0 g.
3. KH_2PO_4 (0.5%)	5.0 g.
4. $MgSO_4$ (0.25%)	2.5 g.
5. Magnesium Citrate (0.25%).	2.5 g.
6. $(NH_4)_2CO_3$ (0.3%)	3.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study constituents essential for growth of the tubercle bacilli.

Variants:

(a) The authors used 0.2% $(NH_4)_2CO_3$ instead of 0.3% and substituted 0.4% ammonium citrate for 0.25% magnesium citrate.

(b) Smith gave the following solution:

1. Distilled water	1000.0 g.
2. $(NH_4)_2CO_3$	3.5 g.
3. KH_2PO_4	1.5 g.
4. $MgSO_4 \cdot 7H_2O$	2.5 g.
5. Glycerol	15.0 g.

(c) Tanner gave the same solution as Smith but used K_3PO_4 instead of KH_2PO_4.

References: Proskauer and Beck (1894 p. 148), Smith (1905 p. 198), Kolle and Wasserman (1912 p. 394), Tanner (1919 p. 68), Klimmer (1923 p. 112).

250. Beijerinck's Glucose Ammonium Sulphate Solution

Constituents:

1. Water	100.0 cc.
2. KH_2PO_4	0.05 g.
3. $(NH_4)_2SO_4$	0.05 g.
4. Glucose	3.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of *Streptothrix chromogena.*

Reference: Beijerinck (1900 p. 10).

251. Doryland's Glucose Ammonium Sulphate Solution

Constituents:

1. Distilled water	500.0 cc.
2. HCl dilute	
3. $MgSO_4$	0.5 g.
4. CaO	0.01 g.
5. $Fe_2(SO_4)_3$	0.01 g.
6. $MnSO_4$	0.01 g.
7. $(NH_4)_2SO_4$	1.00 g.
8. H_2SO_4	
9. H_3PO_4	
10. Glucose	10.0 g.
11. N/ 0.2578 NaOH	
12. N/ 0.6205 KOH	

Preparation:

(1) Dilute HCl so that 1.0 cc. is not quite neutralized by 1.0 cc. of silicate solution made by dissolving 24.0 g. K_2SiO_3 and 8.4 g. Na_2SiO_3 in 500.0 g. distilled water. (Phenolphthalein as indicator.)

(2) Add to the HCl the following salts:

$MgSO_4$	0.5 g.
CaO	0.01 g.
$Fe_2(SO_4)_3$	0.01 g.
$MnSO_4$	0.01 g.
$(NH_4)_2SO_4$	1.0 g.

(3) Standardize (2) against the silicate solution so that 1.0 cc. silicate equivalent to 1.0 cc. (2) using methyl orange as indicator.

(4) Standardize a solution of H_2SO_4 in same way as HCl omitting the salts.

(5) Standardize H_3PO_4 in similar way as HCl omitting salt and using phenolphthalein as indicator.

(6) Mix the acids in the following ratio:

HCl	153.5 cc.
H_2SO_4	77.0 cc.
H_3PO_4	116.0 cc.

(7) Mix equal quantities of N/0.6205 KOH and N/0.2578 NaOH.

(8) 1.0 cc. of (7) should neutralize 1.0 cc. of (6), using phenolphthalein as indicator.

(9) Draw acid and base into separate plugged burettes, allow to stand several hours to sterilize.

(10) Add enough sterile glucose solution to equal amounts of (6) and (7) to give concentration of glucose of 10.0 grams per liter to a mixture of equal amounts of (6) and (7).

Use: General synthetic culture medium.

Reference: Doryland (1916 pp. 146–148).

252. Winogradsky's Glucose Ammonium Sulphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Dextrose	20.0 g.
3. Potassium phosphate	1.0 g.
4. $MgSO_4$	0.2 g.
5. NaCl	small amounts
6. $FeSO_4$	
7. $MnSO_4$	
8. $(NH_4)_2SO_4$	0.01 g.
9. Chalk	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

(2) Adjustment of reaction not specified.

(3) Distribute into Drechsel's flasks with a ground cover.

(4) Add an excess of chalk to each flask.

(5) After sterilization connect the flask with a nitrogen generator and after inoculation replace the air with nitrogen.

Sterilization: Sterilize in an autoclave.

Use: Cultivation of *Clostridium Pastorianum.*

Reference: Winogradsky (1902 p. 49).

253. Gage's Glucose Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2SO_4$	4.0 g.
3. Glucose	1.0 g.
4. KH_2PO_4	2.0 g.
5. $MgSO_4$	1.0 g.
6. Iron sulphate	0.8 g.
7. NaCl	4.0 g.
8. $MgCO_3$	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Distribute in 100.0 cc. lots in 150.0 cc. flasks.

(3) To each flask add 1.0 cc. of a heavy suspension of $MgCO_3$.

Sterilization: Not specified.

Use: To study nitrification by *Nitrosobacter* and *Pseudomonas radicicola.*

Reference: Gage (1910 p. 33).

254. Stoklasa's Glucose Ammonium Sulphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. d-glucose	25.0 g.
3. K_2SO_4	1.0 g.
4. $MgCl_2$	0.5 g.
5. Iron sulphate	0.1 g.
6. $(NH_4)_2SO_4$	
7. One of the following: Monodiferric-phosphate $Fe_2O_3(P_2O_5)_3$ Monodialuminum-phosphate $Al_2O_3P_2O_5 \cdot 8H_2O$	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 2.0 g. nitrogen in the form of $(NH_4)_2SO_4$ in 1.

(2) Add 1.0 g. of one of 7 to (1).

(3) Adjustment of reaction not given.

Sterilization: In the autoclave.

Use: To study the cycle of the phosphate ion in the soil using *Bac. proteus and vulgaris*, *Bac. mesentericus vulgatus.* PO_4 ions were probably formed.

Reference: Stoklasa (1911 p. 479).

255. Percival's Glucose Ammonium Sulphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $(NH_4)_2SO_4$	2.0 g.
3. K_2HPO_4	1.0 g.
4. NaCl	2.0 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.4 g.
7. $MgCO_3$	10.0 g.
8. Dextrose 0.01, 0.03, 1.0 or (3.0%) 0.1, 0.3, 3.0, 10.0 or 30.0 g.	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Distribute in 50.0 cc. into conical flasks.

(3) Add 0.5 g. of basic $MgCO_3$ to each flask.

(4) To each flask add 0.01, 0.03, 0.3, 1.0 or 3.0% glucose.

Sterilization: Not specified.

Use: To study nitrification by bacteria from the soil.

Reference: Percival (1920 p. 144).

256. Lipman, Waksman and Joffe's Glucose Sulphur Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $(NH_4)_3SO_4$	2.0 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4$	0.5 g.
5. KCl	0.5 g.
6. $FeSO_4$	0.01 g.
7. Glucose	10.0 g.
8. Sulphur	10.0 g.
9. $Ca_3(PO_4)_2$	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

(2) Adjust to pH between 6.0 and 6.2.

Sterilization: Sterilize in the steamer the fractional method.

Use: Cultivation of organisms capable of oxidizing sulphur.

Variants: The authors used only 1.0 g. glucose instead of 10.0 g.

Reference: Lipman, Waksman and Joffe (1921 p. 475) taken from (1922 p. 108).

257. Bokorny's Sucrose Ammonium Sulphate Solution (Basic)

Constituents:

1. Distilled water	1000.0 cc.
2. Sucrose	100.0 g.
3. K_2HPO_4	5.0 g.
4. $MgSO_4$	1.0 g.
5. $(NH_4)_2SO_4$	5.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: To study yeast growth.

Variants: Zikes used the modification given below to study volutin formation by wine yeast, *Saccharomyces Frohberg*, *Saccharomyces anamensis*, *Saccharomyces ilicis*, *Saccharomyces*, *Will* (Bajonus) *Pichia membranaefaciens*, *Cholara mycoderma*, *Monilia candida*, *Oidium lactis*, *Endomyces Magnusii.*

1. Distilled water	1000.0 cc.
2. Sucrose	100.0 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4$	0.3 g.
5. $(NH_4)_2SO_4$	2.5 g.

($pH = 5.3718$, $CH = 4.25 \times 10^{-6}$).

Reference: Bokorny (1911 p. 183), Zikes (1922 p. 29).

258. Wellman's Starch Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2SO_4$	2.0 g.
3. KNO_3	1.0 g.
4. K_3PO_4	2.0 g.
5. NaCl	2.0 g.
6. Starch	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add starch to (1). (Amount not given.)

Sterilization: Not specified.

Use: Cultivation of *Glycobacter proteolyticus.*

Reference: Wellman (1912 p. 616).

259. Heller's Starch Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. Sodium phosphate	0.5 g.

3. Ammonium sulphate	0.5 g.
4. Soluble starch	10.0 g.
5. Calcium carbonate	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Adjustment of reaction not specified.

Sterilization: Not specified.

Use: Enrichment of soil anaerobes.

Reference: Heller (1921 p. 453).

260. Heinze's Glycogen Ammonium Sulphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4	2.0 g.
3. $MgSO_4$	0.4 g.
4. $CaCl_2$	0.2 g.
5. Glycogen	10.0 g.
6. $(NH_4)_2SO_4$	0.1 g.
7. $FeCl_3$ (dilute solution)	20 drops

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Cultivation of *Aspergillus* and other molds.

Variants:

(a) The author used 2.5 g. glycogen instead of 10.0 g.

(b) The author used 1.3 g. $(NH_4)_2SO_4$ and 2.5 g. of glycogen instead of 0.1 g. $(NH_4)_2SO_4$ and 10.0 g. of glycogen.

(c) The author used 4.0 g. $MgSO_4$, 2.5 g. glycogen and omitted the $FeCl_3$ solution.

Reference: Heinze (1904 p. 182).

261. McBeth's Cellulose Ammonium Sulphate Solution (Sanborn)

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $MgSO_4$	1.0 g.
4. Na_2CO_3	1.0 g.
5. $(NH_4)_2SO_4$	2.0 g.
6. Cellulose	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add a suitable source of cellulose.

Sterilization: Sterilize in the autoclave at 15 pounds pressure.

Use: Cultivation of *Cellculomonas folia.* The author used maple leaves and filter paper as sources of cellulose. He reported that the presence or absence of an "essential food factor represented by vitamine B (?) exerts a stimulating effect upon the growth in physiological efficiency of *C. folia.*"

Reference: Sanborn (1926 p. 2).

262. Löhnis' Ammonium Sulphate Cellulose Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $(NH_4)_2SO_4$	1.0 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4$	0.5 g.
5. NaCl	trace
6. $CaCO_3$	20.0 g.
7. Cellulose	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Fill tubes with (1).

(3) Add about 0.1 g. of filter paper, cotton wool or straw to each tube.

Sterilization: Not specified.

Use: Cultivation of cellulose decomposers. Author inoculated with rotten dung.

Variants: Cunningham tubed the solution in 15.0 to 20.0 cc. quantities and added a small quantity of finely cut filter paper to each tube. Fermentation tubes with a glass rod inside to hold them above the $CaCO_3$ were added. The medium was used to grow anaerobes.

Reference: Löhnis (1913 p. 93), Cunningham (1924 p. 142).

263. Löhnis' Pectin Ammonium Sulphate Solution

Constituents:

1. Water (tap)	1000.0 cc.
2. $(NH_4)_2SO_4$	0.5 g.
3. K_2HPO_4	0.5 g.
4. $CaCO_3$	20.0 g.
5. Pectin	5.0 g.

Preparation:

(1) Grate and extract about 500.0 g. of carrot roots with warm water containing chloroform.

(2) Treat the residue for about 30 minutes (without warming) with dilute caustic soda

(3) Wash (2) with water.

(4) Extract for 30 minutes with dilute HCl.

(5) Again wash with water.

(6) Dissolve the pectin by adding dilute ammonia and precipitate it from solution by the addition of $CaCl_2$.
(7) Thoroly wash the pectin precipitate with distilled water.
(8) Add 0.1 g. of (7) to a series of flasks.
(9) Dissolve 2, 3 and 4 in 1.
(10) Add 2.0 cc. of (9) to each flask of (8).

Sterilization: Not specified.

Use: Enrichment of *B. amylobacter*.

Reference: Löhnis (1913 p. 92).

264. Beijerinck's Glucose Starch Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose	30.0 g.
3. Starch	1.0 g.
4. KH_2PO_4	0.5 g.
5. $(NH_4)_2SO_4$	0.5 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of *Streptothrix chromogena*. The author found that the medium supported growth very well.

Reference: Beijerinck (1900 p. 11).

265. Proskauer and Beck's Mannitol Glycerol Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. Mannitol (0.6%)	6.0 g.
3. Glycerol (1.5%)	1.5 g.
4. KH_2PO_4 (0.5%)	5.0 g.
5. $MgSO_4$ (0.25%)	2.5 g.
6. $(NH_4)_2SO_4$ (0.2%)	2.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To determine constituents essential for the growth of the tubercle bacillus.

Reference: Proskauer and Beck (1894 p. 147).

266. Proskauer and Beck's Basal Glycerol Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol (1.5%)	15.0 g.
3. KH_2PO_4 (0.5%)	5.0 g.
4. Magnesium citrate (0.25%)	2.5 g.
5. $(NH_4)_2SO_4$ (0.2%)	2.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Add one of the carbohydrates listed to (1).

Sterilization: Not specified.

Use: To study the constituents essential for the growth of the tubercle bacillus.

Added nutrients:

(a) The authors added one of the following carbohydrates.

no added carbon	
raffinose	1.0%
sucrose	1.0%
maltose	1.0%
lactose	1.0%
glucose	1.0%
mannose	1.0%
levulose	1.0%
dulcitol	0.6%
isodulcitol	

(b) Tiffeneau and Marie added 0.6% mannitol as a carbon source.

Reference: Proskauer and Beck (1894 pp. 142–146), Tiffeneau and Marie (1912 p. 48).

267. Doryland's Acetic Acid Ammonium Sulphate Solution

Constituents:

1. Water	500.0 cc.
2. NaOH, N/0.2578	
3. KOH, N/0.6205	
4. HCl	
5. $MgSO_4$	0.5 g.
6. CaO	0.01 g.
7. $Fe_2(SO_4)_3$	0.01 g.
8. $MnSO_4$	0.01 g.
9. $(NH_4)_2SO_4$	1.0 g.
10. H_2SO_4	
11. H_3PO_4	
12. CH_3COOH	

Preparation:

(1) Dilute the HCl so that 1.0 cc. is not quite neutralized by 1.0 cc. of a silicate solution made by dissolving 24.0 g. K_2SiO_3 and 8.4 g. Na_2SiO_3 in 500.0 g. water. Phenolphthalein is used as an indicator.

(2) Add to the HCl the following salts:

$MgSO_4$	0.5 g.
CaO	0.01 g.
$Fe_2(SO_4)_3$	0.01 g.
$MnSO_4$	0.01 g.
$(NH_4)_2SO_4$	1.0 g.

(3) Standardize the resulting HCl solutions against the silicate, using

methyl orange as an indicator, so that 1.0 cc. is equivalent to 1.0 cc. of the silicate solution.

(4) Standardize a solution of H_2SO_4 in the same way as HCl omitting the salts.

(5) Standardize H_3PO_4 and CH_3COOH in a similar manner as HCl omitting the salts and using phenolphthalein as an indicator.

(6) Mix the acids in the following proportion:

HCl	153.5 cc.
CH_3COOH	153.5 cc.
H_2SO_4	77.0 cc.
H_3PO_4	116.0 cc.

(7) Mix equal quantities of N/0.6205 KOH and N/0.2578 NaOH.

(8) 1 cc. of (7) should neutralize 1 cc. of (6) using phenolphthalein as indicator.

(9) Acids and bases are drawn into separate burettes and allowed to stand to sterilize.

(10) Mix equal amounts when ready to use.

Sterilization: Not specified.

Use: As a general synthetic culture medium.

Reference: Doryland (1916 p. 146).

268. Proskauer and Beck's Basal Citrate Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4 (0.5%)	5.0 g.
3. Magnesium citrate (0.25%)	2.5 g.
4. $(NH_4)_2SO_4$ (0.25%)	2.5 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add 1.0% of one of the listed carbohydrates.

Sterilization: Not specified.

Use: To determine the constituents essential for growth of the tubercle bacillus.

Added nutrients: The authors added 1.0% of one of the following carbohydrates:

levulose
mannose
maltose
sucrose
lactose
raffinose
dulcitol 0.6%
isodulcitol 0.6%
mannitol 0.6%

Reference: Proskauer and Beck (1894 pp. 144-146).

269. Peglion's Sucrose Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. Cane sugar	50.0 g.
3. Cream of tartar	1.0 g.
4. $MgSO_4$	0.3 g.
5. $(NH_4)_2SO_4$	0.2 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of *B. oncidii*.

Reference: Peglion (1899 p. 36).

270. Higgins' Succinate Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose	2.5 g.
3. Lactose	2.5 g.
4. Ammonium succinate	2.5 g.
5. $(NH_4)_2SO_4$	1.0 g.
6. KCl	1.5 g.
7. KNO_3	2.5 g.
8. Ammonium phosphate	2.5 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Method not specified.

Use: Cultivation of organisms causing cholera, Picarton cattle disease and other organisms. The author found that this medium gave approximately the same results as ordinary broth.

Reference: Higgins (1898 p. 668).

271. Lockemann's Citrate Ammonium Sulphate Solution

Constituents:

1. Water	1000.0 cc.
2. $NaH_2PO_4 \cdot H_2O$	3.0 g.
3. KH_2PO_4	4.0 g.
4. $(NH_4)_2SO_4$	5.0 g.
5. Magnesium citrate	2.5 g.
6. Glycerin	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) The reaction should be acid titer 1/100 N 3.3.

(3) Distribute in 50.0 cc. lots in flasks.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

Reference: Lockemann (1919 p. 421).

272. Beijerinck's Glucose Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 g.
2. Glucose	20.0 g.
3. K_2HPO_4	0.2 g.
4. $(NH_4)_2HPO_4$	0.2 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: The author found that *Azotobacter chroococcum* utilized ammonium salts readily in this medium.

Reference: Beijerinck (1901 p. 575).

273. Ayer's Glucose Ammonium Phosphate Solution (Caldwell)

Constituents:

1. Distilled water	1000.0 cc.
2. Sodium ammonium phosphate	4.0 g.
3. NaH_2PO_4	2.0 g.
4. Glucose	10.0 g.
5. Marble	

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Prepare a 20% glucose solution in distilled water.
(3) Mix sterile (1) and sterile (2).
(4) Tube in sterile tubes containing a little ground marble.

Sterilization: Method not given.

Use: To study anticolon bacteriophage in a synthetic medium.

Reference: Caldwell (1926 p. 123).

274. Kendall, Day and Walker's Glucose Ammonium Phosphate Solution

Constituents:

1. Redistilled water	1000.0 cc.
2. $(NH_4)_2HPO_4$	4.0 g.
3. NaCl	5.0 g.
4. Glucose	10.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Adjustment of reaction not given.

Sterilization: Not specified.

Use: To study the metabolism of the tubercle bacillus. Alizarin, neutral red and phenolphthalein were used to study changes in reaction; Ziehl-Nielsen stain for staining. Kendall, Walker and Day used the solution for the study of lipase production by the tubercle bacillus. Different esters with clear bacteria free culture broth were incubated for 24 hours; the amount of acid produced (measured in terms of N/50 NaOH), determined lipase production.

References: Kendall, Day and Walker (1914 p. 434), Kendall, Walker and Day (1914 p. 455).

275. Wherry's Levulose Sodium Ammonium Phosphate Solution

Constituents:

1. Redistilled water	1000.0 cc.
2. Levulose	2.0 g.
3. Sodium ammonium phosphate	2.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Sterilize in autoclave (time not given).

Use: To study acid proofness of *B. tuberculosis.* Author reported good growth, but the organisms stain irregularly and are not acid proof.

Reference: Wherry (1913 p. 150).

276. Wolffin's Glucose Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. Dextrose	100.0 g.
3. Potassium phosphate	1.0 g.
4. Ammonium phosphate	10.0 g.
5. $MgSO_4$	0.2 g.
6. $CaCO_3$	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Adjustment of reaction not specified.
(3) Distribute in flasks in 250.0 cc. lots.
(4) Add 1.0 g. $CaCO_3$ to each flask.

Sterilization: Not specified.

Use: To study fermentation by *Bacillus levans.*

Reference: Wolffin (1894 p. 288).

277. Stutzer's Sucrose Ammonium Magnesium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. Sucrose	20.0 g.
3. Potassium phosphate	1.0 g.
4. Ammonium magnesium phosphate (dry) ($MgNH_4PO_4$)	2.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of bacteroids from *Pisum sativum.*

Reference: Stutzer (1900 p. 902).

278. Henneberg's Sucrose Ammonium Phosphate Solution

Constituents:

1. Distilled water	1000.0 g.
2. K_2HPO_4 (0.5%)	5.0 g.
3. $MgSO_4$ (0.2%)	2.0 g.
4. Cane sugar (5.0%)	50.0 g.
5. Ammonium phosphate (0.3%)	3.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of yeast.

Reference: Henneberg (1907 pp. 40–45), (1909 p. 104).

279. Botkin's Starch Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. Ammonium phosphate	0.08 g.
3. Potassium phosphate	0.04 g.
4. $MgSO_4$	0.04 g.
5. $(NH_4)_2SO_4$	0.02 g.
6. Starch, potato	30.0 g.
7. $CaCO_3$	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Add 7 to (1).

Sterilization: Not specified.

Use: To ascertain fermentation of starch by *Bacillus butyricus.* The author found that gas was produced from the starch after 24 hours and that the culture reduced Fehling's solution.

Reference: Botkin (1872 p. 431).

280. Waksman and Carey's Ammonium Phosphate Cellulose Solution

Constituents:

1. Distilled water	100.0 cc.
2. $(NH_4)_2HPO_4$	5.0 g.
3. $MgSO_4$	1.0 g.
4. $FeSO_4$	0.02 g.
5. KCl	1.0 g.
6. Cellulose (filter paper)	4.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Grind filter paper.

(3) Add 1.0 g. ground filter paper to flasks containing 25.0 cc. of sterile (1).

Sterilization: Method not given.

Use: Cultivation of *Spirochaeta cytophaga* and other organisms capable of decomposing cellulose.

Variant: The authors used the medium diluting each flask containing 25.0 cc. of the medium with 150.0 cc. of water.

Reference: Waksman and Carey (1926 p. 92).

281. Percival's Cellulose Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4	1.0 g.
3. Ammonium phosphate	1.0 g.
4. $MgSO_4$	0.5 g.
5. NaCl	a small crystal
6. Filter paper or Blotting paper	3.0 g.
7. $CaCO_3$	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Pour into 250.0 cc. flasks and add 5.0 g. of $CaCO_3$.

(3) Cut about 3.0 g. of filter or blotting paper into small pieces and add to (2).

(4) Inoculate with a piece of fresh horse dung or mud from the bottom of a pond.

(5) Fill the flasks completely and insert a cork thru which passes a bent glass tube, arranged so as to collect any gas given off.

Sterilization: Not specified.

Use: Cultivation of *B. amylobacter,* a cellulose splitting organism. Author reported the production of gas after 3 or 4 weeks.

Reference: Percival (1920 p. 231).

282. Loew's Formaldehyde Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4	2.0 g.
3. $(NH_4)_2HPO_4$	1.0 g.
4. $MgSO_4$	0.1 g.
5. $CaCl_2$	0.1 g.
6. Sodium salt or formaldehyd-sulfurous acid ("Formaldehyschwefligsaurem Natrom")	5.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Isolation of a red bacillus from the air capable of utilizing formic acid as a carbon source.

Reference: Loew (1892 p. 462).

283. Loew's Methyl Alcohol Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. Methyl alcohol	5.0 g.
3. K_2HPO_4	0.5 g.
4. $(NH_4)_2HPO_4$	0.5 g.
5. $MgSO_4$	0.1%

Preparation:

(1) Dissolve 3, 4 and 5 in 1.

(2) After sterilization add 2 to (1).

Sterilization: Method not given.

Use: Isolation of bacillus from the air, producing red pigment and capable of assimilating formic acid.

Reference: Loew (1892 p. 463).

284. Omeliansky's Ethyl Alcohol Ammonium Phosphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $MgSO_4$	0.5 g.
4. Ammonium phosphate	1.0 g.
5. NaCl	traces
6. Ethyl alcohol	10.0 cc.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Distribute into long necked flasks with lateral tubes.

(3) Add a small amount of chalk to each tube.

(4) After sterilization cool.

(5) Add 1.0 part ethyl alcohol to each 100.0 parts medium under aseptic conditions.

Sterilization: Method not specified.

Use: Fermentation of ethyl alcohol with the production of methane.

Reference: Omeliansky (1916 p. 58).

285. Beijerinck's Alcohol Ammonium Phosphate Solution

Constituents:

1. Water (tap)	1000.0 cc.
2. Alcohol	30.0 g.
3. Ammonium phosphate	0.5 g.
4. KCl	0.1 g.

Preparation:

(1) Dissolve 3 and 4 in tap water.

(2) Distribute in 10.0 cc. lots in Frendenreich flasks.

(3) After sterilization add 3.0% by volume of absolute alcohol and sufficient acetic acid to each tube to dissolve the precipitate.

Sterilization: Method not given.

Use: Cultivation of acetic acid bacteria.

References: Beijerinck (1898 p. 214), Janke (1916 p. 6).

286. De Schweinitz's Glycerol Ammonium Phosphate Solution (Gage and Phelps)

Constituents:

1. Distilled water	1000.0 cc.
2. $MgSO_4$ (0.02%)	0.2 g.
3. K_2HPO_4 (0.1%)	1.0 g.
4. Ammonium phosphate (1.0%)	10.0 g.
5. Glycerin (4.0%)	40.0 g.
6. Neutral red 1.0% water solution (1.0%)	10.0 cc.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add 10.0 cc. of a 1.0% water solution of neutral red to (1).

Sterilization: Not specified.

Use: To observe neutral red reaction by colon types. *B. coli* changes the color from red to yellow.

Reference: Gage and Phelps (1902 p. 411).

287. Löwenstein's Glycerol Ammonium Phosphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium phosphate	6.0 g.
3. Glycerol	40.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Method not given.

Use: Cultivation of tubercle bacilli. Tubercle bacilli develop very poorly on this medium. Na, K, Cl or S is necessary for growth.

Variants:

(a) Lockemann specified the use of $(NH_4)_3PO_4 \cdot 3H_2O$ and added 0.6 g. $MgSO_4 \cdot 7H_2O$.

(b) Lockemann specified the use of $(NH_4)_3PO_4 \cdot 3H_2O$ and added 0.6 g.

$MgSO_4 \cdot 7H_2O$ and 3.0 g. $Na_2HPO_4 \cdot H_2O$.

References: Löwenstein (1913 p. 592), Lockemann (1919 p. 421).

288. Capaldi and Proskauer Mannitol Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. Ammonium phosphate (0.2%)	2.0 g.
3. Mannite (0.2%)	2.0 g.
4. NaCl (0.02%)	0.2 g.
5. $MgSO_4$ (0.01%)	0.1 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: To determine acid production by *B. coli*.

Reference: Capaldi and Proskauer (1896 p. 459).

289. Omeliansky's Mannitol Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. Mannitol	20.0 g.
3. Potassium phosphate	1.0 g.
4. Ammonium phosphate	1.0 g.
5. $MgSO_4$	0.5 g.
6. NaCl	0.2 g.
7. Chalk	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Add 20.0 g. of chalk.
(3) After sterilization add chalk to each tube.

Sterilization: Sterilize at 115°C.

Use: To study fermentation of mannitol by *Bact. formicicum*.

Reference: Omeliansky (1903 p. 319).

290. Loew's Formate Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4	2.0 g.
3. $(NH_4)_2HPO_4$	1.0 g.
4. $MgSO_4$	0.1 g.
5. $CaCl_2$	0.1 g.
6. Sodium formate	5.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To show the assimilation of formic acid by a bacillus producing a red pigment isolated from the air. Pure formic acid gave the same results.

Reference: Loew (1892 p. 463).

291. Stutzer's Citrate Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. Ammonium phosphate	2.0 g.
3. $Mg(NO_3)_2$	2.0 g.
4. Citric acid	0.5 g.

Preparation: (1) Dissolve 2, 3, 4 in 1.

Sterilization: Not specified.

Use: Cultivation of Bacteroids from *Pisum sativum*.

Reference: Stutzer (1906 p. 901).

292. Koser's Citrate Sodium Ammonium Phosphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $Na(NH_4)HPO_4 \cdot 4H_2O$ (microcosmic salt)	1.5 g.
3. KH_2PO_4	1.0 g.
4. $MgSO_4$	0.2 g.
5. Sodium citrate	2.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) pH is about 6.7 to 6.9.
(3) Distribute in 5.0 to 8.0 cc. quantities.

Sterilization: In the autoclave.

Use: Differentiation of colon-aerogenes group. *Bact. coli* of fecal origin is unable to utilize citric acid as a source of carbon, while the aerogenes-cloacae types utilize citric acid.

Variants:

(a) Potassium citrate may replace the sodium salt. Ammonium citrate is quite unstable.
(b) A. P. H. A. used from 2.5 to 3.0 g. sodium citrate.

Reference: Koser (1924 p. 63), American Public Health Assoc. (1925 p. 112).

293. Koser's Citrate Ammonium Phosphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. NaCl	5.0 g.
3. $MgSO_4$	0.2 g.
4. $NH_4H_2PO_4$	1.0 g.

5. K_2HPO_4	1.0 g.
6. Sodium citrate	2.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1. (Use 2.77 g. sodium citrate 5$\frac{1}{2}$ H_2O.)

(2) pH is about 6.7 to 6.9.

(3) Distribute in 5.0 to 8.0 cc. quantities.

Sterilization: In the autoclave.

Use: Differentiation of the colon-aerogenes group. *Bact. coli* of fecal origin is unable to utilize citric acid as a source of carbon, while the aerogenes-cloacae types utilize citric acid.

Variants: Potassium citrate may be used instead of sodium citrate. Ammonium citrate is quite unstable.

Reference: Koser (1924 p. 63).

294. Tausz and Peter's Paraffin Magnesium Ammonium Phosphate Solution

Constituents:

1. Water	1000.0 cc.
2. $MgNH_4PO_4$ (0.1%)	1.0 g.
3. $CaSO_4$ (0.01%)	0.1 g.
4. K_2HPO_4 (0.08%)	0.8 g.
5. $FeCl_3$	trace
6. KI	trace
7. Paraffin (amount not specified).	

Preparation: (1) Mix 1, 2, 3, 4, 5, 6 and 7. Complete solution does not take place.

Sterilization: Steam by the Klöcker method on 2 successive days for 30 minutes each day.

Use: Cultivation of *Bacterium aliphaticum, Bacterium aliphaticum liquefaciens,* paraffin bacteria.

Reference: Tausz and Peter (1919 p. 507).

295. Söhngen's Methane Magnesium Ammonium Phosphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	0.5 g.
3. $MgNH_4PO_4$	1.0 g.
4. $CaSO_4$	0.1 g.
5. Methane	

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Flask and inoculate (garden earth, dung water or ditch water may be used for inoculation).

(3) Fill the flask with a known mixture of oxygen and methane.

Sterilization: Not specified.

Use: Isolation and enrichment of *Bacillus methanicus* (utilizing methane).

References: Söhngen (1905 p. 514), Löhnis (1913 p. 105).

296. Laurent's Sucrose Ammonium Phosphate Solution (Pringsheim)

Constituents:

1. Water	1000.0 cc.
2. Sugar	100.0 g.
3. Tartaric acid	1.0 g.
4. KH_2PO_4	0.75 g.
5. $MgSO_4$	0.1 g.
6. $(NH_4)_3PO_4$	5.0 g.

Preparation: Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study the bios requirements of yeast. The author reported that fermentation occurred after 2 months.

Reference: Pringsheim (1906 p. 114).

297. Janke's Alcohol Ammonium Phosphate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	0.4 g.
3. $(NH_4)_2HPO_4$	1.0 g.
4. $MgSO_4 + 7H_2O$	0.4 g.
5. Glycerin	5.0 cc.
6. Succinic acid	1.0 g.
7. Alcohol (3.0%)	30.0 cc.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in distilled water.

(2) Add distilled water to (1) to make 1000.0 cc.

(3) After sterilization add 3.0% by volume of alcohol.

Sterilization: Method not given.

Use: Cultivation of acetic acid bacteria.

Variants: H_3PO_4 or acetic acid may be used instead of succinic acid. The acid is used for the purpose of keeping the materials in solution. Succinic acid does not inhibit growth whereas the other mentioned acids do.

Reference: Janke (1916 p. 7), (1921 p. 91).

298. Wherry's Ammonium Acetate Salt Solution

Constituents:

1. Redistilled water	1000.0 cc.
2. NaCl	1.0 g.
3. KCl	1.0 g.

4. $CaCl_2$	1.0 g.
5. $MgSO_4$	1.0 g.
6. KH_2PO_4	2.0 g.
7. Ammonium acetate	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Adjustment of reaction not specified.

Sterilization: In the autoclave (time not given).

Use: To study acid proofness of *B. tuberculosis*.

Variants:

(a) The KH_2PO_4 may be omitted.

(b) Na_2CO_3 may be substituted for NaCl.

(c) 2.0 g. of potassium acetate and 2.0 g. of calcium acetate may be added.

(d) 2.0 g. of ammonium acetate with 2.0 g. or 1.0 g. of KH_2PO_4 may be used.

Reference: Wherry (1913 pp. 147–148).

299. Harvey's Ammonium Acetate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium acetate	10.0 g.
3. K_2HPO_4	5.0 g.
4. $MgSO_4$	5.0 g.
5. $Ca_3(PO_4)_2$	0.5 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Reference: Harvey (1921–22 p. 103).

300. Proskauer and Beck's Basal Ammonium Tartrate Solution

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4 (0.5%)	5.0 g.
3. $MgSO_4$ (0.25%)	2.5 g.
4. Ammonium tartrate (0.2%)	2.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add one of the carbon sources listed.

Sterilization: Not specified.

Use: To study the constituents essential for growth of the tubercle bacillus.

Added nutrients and variants:

(a) The authors employed the following carbon sources:

mannitol	0.6%
dulcitol	0.6%
isodulcitol	0.6%
sucrose	1.0%
lactose	1.0%
maltose	1.0%
raffinose	1.0%
mannose	1.0%
levulose	1.0%

(b) Besson used a solution containing 10.0 g. ammonium phosphate, 1.0 g. potassium phosphate, 0.2 g. $MgSO_4$ and 0.12 g. $CaCl_2$ per liter and added 2.0% sucrose or glucose.

References: Proskauer and Beck (1894 pp. 144–147), Besson (1920 p. 37).

301. Carapelle's Methylene Blue Ammonium Tartrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium tartrate	10.0 g.
3. Potassium phosphate	10.0 g.
4. $MgSO_4$	5.0 g.
5. Tri-basic calcium phosphate	5.0 g.
6. Methylene blue (1.0% soln.)	several drops

Preparation:

(1) Dissolve 2, 3, 4 and 5 in (1).

(2) Tube after sterilization.

(3) Add several drops of a 1.0% methylene blue solution to each tube.

Sterilization: Method not given.

Use: To determine reduction ability of bacteria.

Variants: One of the following indicators may replace methylene blue:

indigo carmine
lackmoid
litmus
sodium salt of indigo sulphuric acid

Reference: Carapelle (1908 p. 552).

302. Meyers' Basal Ammonium Tartrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium tartrate	10.0 g.
3. Anhydrous sodium sulphate	10.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1. Flask.

(2) Adjustment of reaction not given.

(3) After sterilization suspend a sterile strip of filter paper soaked in 10.0% lead acetate solution in each flask.

Sterilization: Autoclave at 15 pounds pressure for 10 minutes.

Use: To determine H_2S production by colon-typhoid group. No growth of culture studied under aerobic condition.

Modification: The author added 10.0 g. glucose.

Reference: Meyers (1920 p. 248).

303. Naegeli's Ammonium Tartrate Solution (Smith)

Constituents:

1. Water	1000.0 cc.
2. Ammonium tartrate	10.0 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4$	0.2 g.
5. $CaCl_2$	0.1 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Variants:

(a) Harvey used 0.12 g. $CaCl_2$ instead of 0.1 g.

(b) Harvey used 10.0 g. K_2HPO_4 instead of 1.0 g., 10.0 g. $MgSO_4$ instead of 0.2 g., and 0.5 g. $Ca_3(PO_4)_2$ instead of 0.1 g. $CaCl_2$.

(c) Harvey used 5.0 g. KH_2PO_4, 0.5 g. calcium phosphate instead of $CaCl_2$ and 5.0 g. $MgSO_4$ instead of 0.2 g.

(d) Tanner, Devereux and Higgins used a similar medium with 0.12 g. $CaCl_2$ for the cultivation of yeast and yeast-like fungi.

References: Smith (1905 p. 197), Harvey (1921–22 p. 103), Tanner, Devereux and Higgins (1926 p. 62).

304. Cohn's Ammonium Tartrate Solution (Smith)

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4	5.0 g.
3. $MgSO_4$	5.0 g.
4. Ammonium tartrate	10.0 g.
5. KCl	0.5 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: General culture medium.

Variants:

(a) Roux and Rochaix specified the use of 10.0 g. potassium phosphate instead of 5.0 g. KH_2PO_4 and used 0.5 g. $Ca_3(PO_4)_2$ instead of KCl.

(b) Kolle and Wassermann did not specify the type of potassium phosphate used, and substituted 0.5 g. $Ca_3(PO_4)_2$ for KCl.

(c) Klimmer omitted the KCl.

References: Smith (1905 p. 197), Roux and Rochaix (1911 p. 103), Kolle and Wassermann (1912 p. 7), Löhnis (1913 p. 44), Tanner (1919 p. 63), Besson (1920 p. 37), Dopter and Sacquépée (1921 p. 121), Giltner (1921 p. 369), Klimmer (1923 p. 172).

305. Nicolle and Zia Bey's Ammonium Lactate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium lactate	10.0 g.
3. $MgSO_4$	2.5 g.
4. Potassium phosphate	0.0 or 5.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) 5.0 g. of potassium phosphate may or may not be added.

Sterilization: Not specified.

Use: To study pigment and fluorescence production by *Bacillus pyocyaneus*. Phosphate hastened and increased pigment and fluorescence production.

Reference: Nicolle and Zia Bey (1896 p. 671).

306. Braun and Cahn-Bronner's Ammonium Lactate Solution

Constituents:

1. Water	1000.0 g.
2. NaCl	5.0 g.
3. KH_2PO_4	2.0 g.
4. Ammonium lactate	6.0 g.

Preparation:

(1) Dissolve 2 and 3 in about 75.0 cc. of water.

(2) Neutralize (1) with sodium carbonate.

(3) Dissolve 4 in 25.0 cc. water.

(4) Boil (2) and (3) for 1 hour to sterilize.

(5) When cool mix the two solutions.

(6) Neutralize to litmus by the addition of sterile N/1 soda solution and then add 0.7 cc. sterile N/1 soda solution.

Sterilization: See step (4) under preparation.

Use: To study food requirements for colon typhoid group. Authors reported that colon bacillus and para typhoid B become strict aerobes in this medium.

Variants: Branham used 0.2% K_2HPO_4 instead of KH_2PO_4 and adjusted the reaction so that the final pH be between 6.8 and 7.2. He used the medium for toxin production by *Bacterium enteritidis.*

References: Braun and Cahn-Bronner (1921 pp. 4, 197), Branham (1925 p. 299).

307. Löhnis' Ferric Ammonium Citrate Solution

Constituents:

1. Water	1000.0 cc.
2. Ferric ammonium citrate (0.05%)	0.5 g.

Preparation:

(1) Dissolve 0.05% ferric ammonium citrate in water.

(2) Distribute in test tubes. Fill the tubes.

Sterilization: Not specified.

Use: Cultivation of iron bacteria.

Variants: Brussoff cultivated *Ferribacterium duplex* on a medium prepared as follows. He reported that growth appeared after several weeks as a very thin yellow membrane.

(1) Boil tap water.

(2) Filter.

(3) Dissolve 0.05% ferric ammonium citrate in (2).

(4) Distribute in 50.0 cc. lots in small Erlenmeyer flasks.

(5) Sterilize in the autoclave for 15 to 20 minutes under 1.5 atmospheres pressure.

References: Löhnis (1913 p. 116), Brussoff (1916 p. 551).

308. Gessard's Ammonium Succinate Solution

Constituents:

1. Water	1000.0 cc.
2. Ammonium succinate	10.0 g.
3. Potassium phosphate	5.0 g.
4. $MgSO_4$	2.5 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: To study pigment and fluorescence production by *Bacillus pyocyaneus.* Also used for the cultivation of tubercle bacilli.

Variants:

(a) The author used 1.0 g. ammonium succinate, specified the use of 5.0 g. of Na_2HPO_4 or K_2HPO_4 and added 1.25 g. $CaCl_2$ to the solution.

(b) Gessard and Vaudremer specified the use of K_2HPO_4, used 5.0 g. ammonium succinate and added 1.25 g. $CaCl_2$ for the cultivation of tubercle bacilli.

References: Gessard (1892 p. 809), (1901 p. 818); Gessard and Vaudremer (1922 p. 1012).

309. Kuntze's Ammonium Succinate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4	10.0 g.
3. $MgCl_2$	1.0 g.
4. Ammonium succinate	10.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: To study pigment production. Author reported that when traces of H_2SO_4 were added to the medium pigment was produced, otherwise no pigment was formed.

Variants: The author used 1.0 g. K_2HPO_4 instead of 10.0 g. KH_2PO_4, and from 1.0 to 2.0 g. $MgSO_4$ instead of 1.0 g. $MgCl_2$. The solution was adjusted to neutrality. *Bacillus prodigiosus* did not produce pigment on this medium.

Reference: Kuntze (1907 p. 306).

310. Fermi's Basal Ammonium Succinate Solution

Constituents:

1. Water	1000.0 cc.
2. Ammonium succinate (0.5 to 1.0%)	5.0 to 10.0 g.
3. KH_2PO_4 (0.5%)	5.0 g.
4. $MgSO_4$ (0.5%)	5.0 g.
5. K_3PO_4 (0.05%)	0.5 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add one of the added nutrients.

Sterilization: Not specified.

Use: Cultivation of *Schizomycetes.*

Added nutrients: The author added 5.0% glycerol or 5.0% sucrose.

Reference: Fermi (1892 p. 26).

311. Fischer's Glucose Ammonium Tartrate Solution (Müller)

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4 (0.1%)	1.0 g.
3. $MgSO_4$ (0.02%)	0.2 g.

4. $CaCl_2$ (0.01%)	0.1 g.
5. Dextrose (1.0%)	10.0 g.
6. Ammonium tartrate (amount not given).	

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of *Bact. Güntheri.*

Reference: Müller (1907 p. 472).

312. Nägeli's Sucrose Ammonium Tartrate Solution (Fermi)

Constituents:

1. Water	1000.0 cc.
2. Ammonium tartrate	1.0 g.
3. KH_2PO_4	1.0 g.
4. $MgSO_4$	0.2 g.
5. $CaCl_2$	2.1 g.
6. Sucrose	50.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of *Schizomycetes.*

Reference: Fermi (1892 p. 26).

313. Groenewege's Sucrose Ammonium Tartrate Solution

Constituents:

1. Water	1000.0 cc.
2. Saccharose	20.0 g.
3. Sodium ammonium tartrate.	1.0 g.
4. K_2HPO_4	0.5 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Invertase production by *Phytobacter lycopersicum n. sp.* (causing tomato rot). Reducing sugars are found to be present when tested with Fehling's solution.

Reference: Groenewege (1913 p. 28).

314. Fermi and Montesano's Sucrose Ammonium Tartrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium tartrate	0.5 g.
3. Potassium phosphate	0.5 g.
4. $MgSO_4$	0.5 g.
5. Calcium phosphate	0.05 g.
6. Sucrose	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Distribute into flasks.

Sterilization: Not specified.

Use: To study inversion of sugar in synthetic medium by bacteria, yeasts and molds.

Variants: Authors substituted 5.0 g. glycerol for sucrose.

Reference: Fermi and Montesano (1895 p. 547).

315. Proskauer and Beck's Glycerol Ammonium Lactate Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol (1.5%)	15.0 g.
3. KH_2PO_4 (0.5%)	5.0 g.
4. $MgSO_4$ (0.25%)	2.5 g.
5. Ammonium lactate (0.5%)	5.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: To study the constituents essential for growth of the tubercle bacillus.

Variants:

(a) The authors added 1.0% glucose, 0.25% K_2SO_4 and 0.12% NaCl.
(b) The authors added 0.3% $(NH_4)_2SO_4$ and omitted 0.25% $MgSO_4$.
(c) Gessard and Vaudremer used 5.0% glycerol and added 0.125% $CaCl_2$.

References: Proskauer and Beck (1894 pp. 142, 148), Gessard and Vaudremer (1922 p. 1012).

316. Stutzer and Hartleb's Glycerol Ammonium Citrate Solution

Constituents:

1. Water	1000.0 cc
2. Ammonium citrate	1.0 g.
3. Glycerol	10.0 g.
4. Potassium phosphate	1.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of a bacterium from a case of foot and mouth disease. The authors report that the medium was strongly clouded after about 3 weeks.

Reference: Stutzer and Hartleb (1897 p. 382).

317. Kuntze's Glycerol Ammonium Succinate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $MgSO_4$	2.0 g.
3. KH_2PO_4	1.0 g.

4. Glycerol (double distilled Merck)	20.0 g.
5. Ammonium succinate	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Adjust the reaction to neutral to litmus.

Sterilization: Method not given.

Use: To study pigment production by *Aspergillus niger* and *Bacillus prodigiosus*.

Reference: Kuntze (1907 p. 303).

318. Wherry's Glycerol Ammonium Acetate Solution

Constituents:

1. Redistilled water	1000.0 cc.
2. NaCl	1.0 g.
3. KCl	1.0 g.
4. $CaCl_2$	1.0 g.
5. $MgSO_4$	1.0 g.
6. Ammonium acetate	5.0 g.
7. KH_2PO_4	2.0 g.
8. Glycerin	50.0 g.

(Salts to be Kahlbaum's c.p.)

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

(2) Adjustment of reaction not given.

Sterilization: Sterilize in autoclave, time not given.

Use: To study acid proofness of *B. tuberculosis*.

Variants: The author substituted $NaCO_3$ for NaCl.

Reference: Wherry (1913 p. 147).

319. Proskauer and Beck's Basal Glycerol Ammonium Tartrate Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol (1.5%)	15.0 g.
3. KH_2PO_4 (0.5%)	5.0 g.
4. $MgSO_4$ (0.25%)	2.5 g.
5. Ammonium tartrate (0.4%)	4.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add additional carbon compounds as desired.

Sterilization: Not specified.

Use: To study the constituents essential for the growth of the tubercle bacillus.

Added nutrients and modifications: The authors suggested the following modifications:

Use of 0.2% ammonium tartrate with the addition of one of the following:

mannitol	0.6%
glucose	1.0%
mannose	1.0%
raffinose	1.0%
maltose	1.0%
lactose	1.0%
sucrose	1.0%
dulcitol	1.0%
isodulcitol	1.0%
magnesium citrate	1.0%
$(NH_3)_2CO_3$	0.3%
levulose	1.0%
K_2SO_4	0.25%
NaCl	0.12%
glucose	1.0%

Reference: Proskauer and Beck (1894 pp. 144–148).

320. Uschinsky's Glycerol Ammonium Lactate Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol	40.0 to 50.0 g.
3. NaCl	5.0 to 7.0 g.
4. $CaCl_2$	0.1 g.
5. $MgSO_4$	0.2 g.
6. K_2HPO_4	1.0 g.
7. Ammonium lactate	10.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: General synthetic culture medium.

Variants: Gratia suggested the following solution adjusted to pH = 7.4:

1. Water	1000.0 g.
2. Glycerol	30.0 cc.
3. NaCl	5.0 g.
4. $CaCl_2$	0.1 g.
5. $MgSO_4$	0.2 g.
6. K_2HPO_4	2.0 g.
7. Ammonium lactate	12.0 g.

Reference: Uschinsky (1893 p. 316), Gratia (1922 p. 295), Brieger and Cohn (1893 p. 9).

321. Fermi and Montesano's Glycerol Ammonium Tartrate Solution

Constituents:

1. Distilled water	100.0 cc.
2. Ammonium tartrate	0.5 g.
3. Potassium phosphate	0.5 g.
4. $MgSO_4$	0.5 g.

5. Calcium phosphate	0.05 g.
6. Glycerol	5.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Distribute into flasks.

Sterilization: Not specified.

Use: Cultivation of bacteria, yeast and molds.

Reference: Fermi and Montesano (1895 p. 547).

322. Gottheil's Ammonium Tartrate Solution No. VI

Constituents:

1. Distilled water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.3 g.
5. NaCl	0.1 g.
6. Ammonium tartrate	10.0 g.
7. Glycerol	10.0 g.
8. Sucrose	5.0 g.
9. Iron	trace

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

Sterilization: Not specified.

Use: General culture medium. Author used organisms from the soil, and bacteria found on roots and rhizomes.

Reference: Gottheil (1901 p. 432).

323. MacKensie's Ammonium Tartrate Solution (Smith)

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium tartrate acid	1.5 g.
3. KH_2PO_4	2.5 g.
4. K_2SO_4	1.5 g.
5. NaCl	0.5 g.
6. Glucose	5.0 g.
7. Lactose	5.0 g.
8. Glycerol	15.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.
(2) Adjust alkaline to phenolphthalein.

Sterilization: Not specified.

Use: General culture medium.

Variants: Tanner used 2.5 g. acid ammonium phosphate and 1.5 g. KH_2PO_4 instead of amounts given by Smith.

Reference: Smith (1905 p. 198), Tanner (1919 p. 68).

SUBGROUP I-C. SECTION 3

Liquid media with constituents of known chemical composition. Nitrogen supplied as nitrites; carbon organic.

A_1. Organic carbon supplied only as carbohydrates.
A_2. Organic carbon supplied as alcohols.
Stutzer and Hartleb's Glycerol Nitrite Solution 324
Beijerinck and van Delden's Mannitol Nitrite Solution 325
A_3. Organic Carbon supplied only as organic acids.
Beijerinck and van Delden's Acetate Nitrite Solution 326
Beijerinck and van Delden's Malate Nitrite Solution 327

324. Stutzer and Hartleb's Glycerol Nitrite Solution

Constituents:

1. Water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. NaCl	1.0 g.
4. $MgSO_4$	0.5 g.
5. KCl	0.1 g.
6. Glycerol	
7. $NaNO_2$	

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Distribute in 200.0 cc. lots.
(3) Prepare a 2.0% solution of sodium nitrite.
(4) After sterilization of (2) and (3) add 5.0 cc. of (3) under 4 drops of glycerin to each 200.0 cc. lot of (3) under aseptic conditions.
(5) Stopper with a double hole rubber stopper so air may be bubbled thru the medium.

Sterilization: Not specified.

Use: To study oxidation of nitrites. The author found that after 14 days neither nitrite nor nitrate test could be obtained. Nitrogen was in organic form. If more $NaNO_2$ be added after 3 days nitrate was present. Air freed of CO_2 was bubbled thru the medium.

Reference: Stutzer and Hartleb (1897 p. 311).

325. Beijerinck and van Delden's Mannitol Nitrite Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	0.5 g.
3. $NaNO_3$	0.5 g.
4. Mannitol	20.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: To study denitrification by *B. subtilis, B. mesentericus* and *Azotobacter chroococcum.* The authors reported that *B. subtilis* did not produce ammonia while the other organisms gave ammonia.

Reference: Beijerinck and van Delden (1902 p. 41).

326. Beijerinck and van Delden's Acetate Nitrite Solution

Constituents:

1. Distilled water	1000.0 cc.
2. KCl	0.2 g.
3. K_2HPO_4	0.2 g.
4. KNO_2	1.0 g.
5. Sodium acetate	0.2 g.
6. Beijerinck and van Delden's basic salt solution (See Med. 25)	10 drops

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add 1 drop of Beijerinck and van Delden's Basic Salt Solution (see Med. 25) to each 100.0 cc. of 1.

(3) Reaction to be slightly alkaline.

Sterilization: Not specified.

Use: Cultivation of *Bacillus oligocarbophilus.* The organism grew as a dry thin snow white scum.

Reference: Beijerinck and van Delden (1903 p. 41).

327. Beijerinck and van Delden's Malate Nitrite Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	0.5 g.
3. $NaNO_2$	0.5 g.
4. Calcium malate	20.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: To study denitrification by *B. subtilis, B. mesentericus* and *Azotobacter chroococcum.* The authors reported that NH_3 was formed only by *Azotobacter chroococcum.*

Reference: Beijerinck and van Delden (1902 p. 41).

SUBGROUP I-C. SECTION 4

Liquid media or basal solutions of known chemical composition. Nitrogen supplied as nitrates; carbon organic.

A_1. Incomplete or basal solutions, employed with the addition of other nutrients.
  Stutzer's Basal Citrate Nitrate Solution.... 328
  Mortensen's Basal Nitrate Salt Solution.... 329
A_2. Complete media.
B_1.* Only one type of organic carbon supplied.
C_1.* Organic carbon supplied as carbohydrates.
D_1. Monosaccharides, only, added.
E_1. Containing inorganic salts of monovalent cations only.
  Stutzer's Glucose Nitrate Solution.. 330
  Buhlert and Fickendey's Glucose Nitrate Solution.... 331
E_2. Containing inorganic salts of mono and di valent cations.
F_1. Primarily for the cultivation of bacteria.
  Beijerinck and Minkman's Glucose Nitrate Solution.... 332
  Maze's Glucose Nitrate Solution.... 333
  Fischer's Glucose Nitrate Solution (Müller).... 334
  Committee S. A. B. Glucose Nitrate Solution.... 335
  Heraeus' Glucose Nitrate Solution.. 336
  Migula's Glucose Nitrate Solution.. 337
  Wherry's Levulose Nitrate Solution. 338
F_2. Not primarily for cultivation of bacteria.
  Went's Glucose Nitrate Solution.... 339
  Charpentier's Glucose Nitrate Solution.... 340
E.$_2$ Inorganic salts of mono, di and trivalent cations added.
  Doryland's Glucose Nitrate Solution. 341
D_2. Disaccharides, only, added.
  Bokorny's Sucrose Nitrate Solution. 342
  Stoklasa and Vitek's Sucrose Nitrate Solution.... 343
  Czapek's Sucrose Nitrate Solution.. 344

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* See page 93 for B_2 and C_2.

D_3. Polysaccharides, only, added.

Gerlach and Vogel's Cellulose Nitrate Solution	345
Dubos' Cellulose Sodium Nitrate Solution	346
Krainsky's Starch Nitrate Solution	347
Löhnis' Cellulose Nitrate Solution	348

C_2. Organic carbon supplied only as alcohols.

D_1. Monoatomic alcohols, only, added.

Beijerinck and Minkman's Alcohol Nitrate Solution	349
Bokorny's Alcohol Nitrate Solution	350

D_2. Polyatomic alcohols, only, added.

Kuhne's Glycerol Nitrate Solution (Proskauer and Beck)	351
Weissenberg's Glycerol Nitrate Solution	352
Lantzsch's Glycerol Nitrate Solution	353
Stoklasa's Mannitol Nitrate Solution	354

C_3. Organic carbon supplied as acids or their salts.

Doryland's Acetic Acid Nitrate Solution	355
Giltay and Aberson's Citrate Nitrate Solution (Murray)	356
Percival's Tartrate Nitrate Solution	357

B_2. More than one type of organic carbon supplied.

C_1. Carbon added as carbohydrates and alcohols.

Gottheil's Nitrate Solution No. VII	358

C_2. Carbon added as carbohydrates and acids.

Burri and Stutzer's Nitrate Solution	359
Kuntze's Nitrate Solution	360
Giltay and Aberson's Nitrate Solution (Stoklasa and Vitek)	361
Giltay's Sucrose Nitrate Solution (Fred)	362
Behrens' Cellulose Nitrate Solution	363

C_3. Carbon added as alcohols and organic acids.

Maassen's Malic Acid Nitrate Solution (Stoklasa and Vitek)	364
Maassen's Stearate Nitrate Solution (Vierling)	365

328. Stutzer's Basal Citrate Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. Potassium citrate	10.0 g.
3. KNO_3	3.0 g.
4. K_2HPO_4	1.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add one of the organic nitrogen compounds given in added nutrients.

(3) Reaction to be slightly alkaline.

(4) Distribute in 10.0 cc. lots.

Sterilization: Method not given.

Use: To study denitrification by *B. agilis*, *B. nitrovorus*, *B. Stutzeri*, *B. Hartlebi*. Author found that nitrate reaction disappeared after 6 to 8 days. When peptone or meat extract was added *B. Stutzeri* and *B. Hartlebi* decomposed all the nitrate in 3 days. *B. nitrovorus* and *B. agilis* after 6 to 7 days.

Added nutrients: Author suggested the addition of peptone or Schülke and Myers beef extract, or a mixture of the two. Amounts not specified.

Variants: The author used the basic solution without additions.

Reference: Stutzer (1901 p. 85).

329. Mortensen's Basal Nitrate Salt Solution

Constituents:

1. Water	1000.0 cc.
2. KNO_3 (0.5%)	5.0 g.
3. Cobalt salt	

Preparation:

(1) Dissolve 2 and 10.0% of one of the carbon sources listed under added nutrients in 1.

(2) Add cobalt salts in varying amounts from 4 to 1/32%.

Sterilization: Not specified.

Use: To determine the toxic properties of cobalt salts for *Aspergillus niger*. The author found the toxic properties of cobalt were different in different media. A 0.5% cobalt chloride solution in a liquid medium was as toxic as 1.0% cobalt chloride in a gelatin medium.

Added nutrients: The author added 10.0% of one of the following to the basic solution:

glucose
sucrose
glycerol

Reference: Mortensen (1909 p. 523).

330. Stutzer's Glucose Nitrate Solution

Constituents:
1. Water
2. Glucose
3. KNO_3

Preparation:
(1) Dissolve 2 and 3 in 1. (Amounts not specified.)
(2) Reaction to be slightly alkaline.
(3) Distribute in 10.0 cc. lots.

Sterilization: Method not given.

Use: To study denitrification by *B. agilis*, *B. nitrovorus*, *B. Stutzeri* and *B. Hartlebi*. Nitrate was not reduced.

Variants: Beijerinck and Minkman used a 2.0% glucose with 1.0% KNO_3 solution to study denitrification. They found a butyric acid fermentation took place when inoculated with soil.

Reference: Stutzer (1901 p. 84), Beijerinck and Minkman (1910 p. 35).

331. Buhlert and Fickendey's Glucose Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose (1.5%)	15.0 g.
3. $NaNO_3$ (0.3%)	3.0 g.
4. Potassium phosphate (0.1%)	1.0 g.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Distribute in 10.0 cc. lots.
(3) Inoculate each tube with 5.0 cc. soil water.

Sterilization: Not specified.

Use: To study denitrification.

Variants:
(a) Barthel specified the use of 0.1% K_3PO_4.
(b) Percival and Harvey specified the use of tap water and used 0.1% K_2HPO_4.

References: Buhlert and Fickendey (1906 p. 402), Barthel (1910 p. 118), Percival (1920 p. 202), Harvey (1921–22 p. 108).

332. Beijerinck and Minkman's Glucose Nitrate Solution

Constituents:

1. Water	100.0 cc.
2. Glucose	2.0 g.
3. Chalk	2.0 g.
4. KNO_3	1.0 g.
5. NH_4Cl	0.05 g.
6. K_2HPO_4	0.05 g.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Inoculate with soil and incubate in a closed flask.

Sterilization: Not specified.

Use: To study denitrification. The authors found that 53.0% CO_2, 32.0% H_2 and 15.0% N_2 were formed if nitrate was present. Without nitrate 50.0% of the gas formed was CO_2 and 50.0% H_2.

Reference: Beijerinck and Minkman (1910 p. 35).

333. Maze's Glucose Nitrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $MgSO_4$	0.5 g.
4. $FeSO_4$	0.02 g.
5. NaCl	0.02 g.
6. $CaCO_3$	1.0 g.
7. Glucose	20.0 g.
8. $NaNO_3$	5.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: To study denitrification by *Bacillus denitrificans*. The author reported the formation of nitrous acid in 24 hours.

Variants: Percival used the following solution to study denitrification by *B. mycoides:*

1. Water	1000.0 g.
2. K_2HPO_4	5.0 g.
3. $MgSO_4$	2.5 g.
4. NaCl	2.5 g.
5. Glucose	2.0 g.
6. $NaNO_3$	2.0 g.

References: Maze (1911 p. 370), Percival (1921 p. 112).

334. Fischer's Glucose Nitrate Solution (Müller)

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4 (0.1%)	1.0 g.
3. $MgSO_4$ (0.02%)	0.2 g.
4. $CaCl_2$ (0.01%)	0.1 g.
5. Dextrose (1.0%)	10.0 g.
6. KNO_3 (amount not specified).	

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study denitrification by *Bacterium*

Güntheri, Bact. Hartlebi, Bac. pyocyaneous and *Bac. fluorescens.*

Variants: von Caron used the following solution to study denitrification:

1. Water	1000.0 cc.
2. Glucose	1.0 g.
3. KNO_3	0.1 or 3.0 g.
4. $MgSO_4$	2.0 g.
5. K_2HPO_4	2.0 g.
6. $CaCl_2$	0.2 g.

References: Müller (1907 p. 472), von Caron (1912 p. 96).

335. Committee S. A. B. Glucose Nitrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $CaCl_2$	0.5 g.
3. Nitrate (kind not given)	1.0 g.
4. Glucose	10.0 g.
5. K_2HPO_4	0.5 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Method not given.

Use: To study reduction of nitrates. Test for nitrite with sulphanilic acid and α-naphthylamine and for ammonia production by Thomas' method.

Variants: Committee S. A. B. specified the use of 1.0 g. KNO_3.

Reference: Committee S. A. B. (1922 p. 525), (1923 p. 27).

336. Heraeus' Glucose Nitrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Potassium phosphate	0.1 g.
3. $MgSO_4$	0.2 g.
4. $CaCl_2$	0.01 g.
5. Glucose	0.8 g.
6. $Ca(NO_3)_2$	0.8 g.
7. $(NH_4)_2CO_3$	

Preparation:

(1) Dissolve 1.0 g. potassium phosphate, 0.2 g. $MgSO_4$ and 0.1 g. $CaCl_2$ in 1000.0 g. distilled water.

(2) Steam for 1 hour.

(3) Allow to stand.

(4) Filter and boil again.

(5) Prepare a solution of $Ca(NO_3)_2$ by treating a weighed amount of $CaCO_3$ with dilute HNO_3 the solutions being warm. Be sure that there is no free HNO_3 in solution. There should be some undissolved salt present. Then add some more $CaCO_3$. This removes all traces of HNO_3.

(6) Steam in a weighted porcelain dish, until a solution is obtained containing 1 part $Ca(NO_3)_2$ to five parts water.

(7) Filter and store.

(8) Prepare solutions of dextrose and $(NH_4)_2CO_3$ the ratio of 1:15.

(9) Mix 25.0 cc. (4), 1.0 cc. of the dextrose solution, 2 drops of the $(NH_4)_2CO_3$ solution and 2 drops of (7).

(10) Dilute the 250.0 cc. with distilled water. A liter of this solution contains:

potassium phosphate	0.1 g.
$MgSO_4$	0.02 g.
$CaCl_2$	0.01 g.
dextrose	0.8 g.
$Ca(NO_3)_2$	0.8 g.

(11) Distribute in 50.0 cc. flasks that have previously been plugged and sterilized.

Sterilization: Heat the solution and the flasks in the steamer for 30 minutes.

Use: Culture medium for soil, water and air forms.

Reference: Heraeus (1896 p. 216).

337. Migula's Glucose Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. KNO_3	10.0 g.
3. Magnesium phosphate	2.0 g.
4. $CaCl_2$	1.0 g.
5. K_2HPO_4	1.0 g.
6. Glucose	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Tube.

Sterilization: Boil in the steamer on two successive days.

Use: Differentiation of typhoid bacilli from *B. coli*.

Reference: Migula (1901 p. 20).

338. Wherry's Levulose Nitrate Solution

Constituents:

1. Redistilled water	1000.0 cc.
2. Na_2CO_3	1.0 g.
3. KCl	1.0 g.
4. $CaCl_2$	1.0 g.
5. $MgSO_4$	1.0 g.
6. KNO_3	4.0 g.

7. K_2HPO_4	2.0 g.
8. Levulose	0.5 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

(2) Adjustment of reaction not given.

Sterilization: Sterilize in autoclave, time not given.

Use: To study acid proofness of *B. tuberculosis.* Luxuriant growth with K_2HPO_4, but no growth without K_2HPO_4. Acid fast properties of organism not given.

Reference: Wherry (1913 p. 151).

339. Went's Glucose Nitrate Solution

Constituents:

1. Water	1000.0 g.
2. Glucose (Commercial)	50.0 g.
3. KNO_3	5.0 g.
4. KH_2PO_4	1.0 g.
5. $MgSO_4$	0.5 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Adjustment of reaction not specified.

Sterilization: Method not given.

Use: Cultivation of *Monilia sitophila (Mont) Sacc.* Organism seems to grow best in damp atmosphere. Also grows well on rice, arachis seeds, bread, roots of *Daucus carota,* milk, etc.

Reference: Went (1901 p. 546).

340. Charpentier's Glucose Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4$	1.0 g.
3. K_2HPO_4	2.0 g.
4. KNO_3	2.0 g.
5. $CaNO_3$	0.05 g.
6. $FeSO_4$	trace
7. Glucose	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Heat at 120°C.

(3) Filter.

Sterilization: Sterilize at 120°C.

Use: To determine nitrate reduction by Algae, *Cystococcus humicola.* The author reported that the total nitrogen content is raised after 13 days incubation.

Variant: The author omitted the 2.0 g. KNO_3 and added 1.0 g. of $Ca(NO_3)_2$ instead of only 0.05 g.

Reference: Charpentier (1903 p. 327).

341. Doryland's Glucose Nitrate Solution

Constituents:

1. Distilled water	500.0 cc.
2. HCl Dilute	
3. $MgSO_4$	0.5 g.
4. CaO	0.01 g.
5. $Fe_2(SO_4)_3$	0.01 g.
6. $MnSO_4$	0.01 g.
7. HNO_3 (amount not given)	
8. H_2SO_4	
9. H_3PO_4	
10. Glucose	5.0 g.
11. N/0.2578 NaOH	
12. N/0.6205 KOH	

Preparation:

(1) Dilute HCl so that 1.0 cc. is not quite neutralized by 1.0 cc. of silicate solution made by dissolving 24.0 g. K_2SiO_3 and 8.4 g. Na_2SiO_3 in 500.0 g. distilled water. Phenolphthalein as indicator.

(2) Add to HCl the following salts.

$MgSO_4$	0.5 g.
CaO	0.01 g.
$Fe_2(SO_4)_3$	0.01 g.
$MnSO_4$	0.01 g.
HNO_3 (amount not specified).	

(3) Standardize (2) against silicate solution so that 1.0 cc. is equivalent to 1.0 cc. using methyl orange as indicator.

(4) Standardize a solution of H_2SO_4 in same way as HCl omitting the salts.

(5) Standardize H_3PO_4 in similar manner as HCl omitting the salts and using phenolphthalein as indicator.

(6) Mix the acids in the following ratio:

HCl	153.5 cc.
H_2SO_4	77.0 cc.
H_3PO_4	116.0 cc.

(7) Mix equal quantities of N/0.6205 KOH and N/0.2578 NaOH.

(8) 1.0 cc. of (7) should neutralize 1.0 cc. of (6) using phenolphthalein as indicator.

(9) Draw acid and base solution in separate burettes and allow to stand several hours to sterilize.

(10) Add enough sterile glucose solution to a mixture of equal parts of (6) and (7) to give 10 g. of glucose per liter solution.

Sterilization: Method not given.

Use: A general synthetic medium.

Reference: Doryland (1916 pp. 146–148).

342. Bokorny's Sucrose Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. KNO_3 (0.4%)	4.0 g.
3. Sucrose (10.0%)	100.0 g.
4. Phosphoric acid (P_2O_5) (0.13%)	1.3 g.

Preparation: (1) Dissolve 2, 3, 4 in 1.

Sterilization: Not specified.

Use: Cultivation of molds.

Variants: Böttger used 0.1% K_2HPO_4 instead of phosphoric acid and used 0.2, 0.5 or 1.0% KNO_3 to determine the toxicity of nitrates for yeast. He reported that nitrate presence had little effect on loss of weight, measured in grams.

Reference: Bokorny (1917 p. 316), Böttger (1921 p. 224).

343. Stoklasa and Vitek's Sucrose Nitrate Solution

Constituents:

1. Water	
2. K_2HPO_4	1.25 g.
3. K_2SO_4	0.2 g.
4. $CaCl_2$	0.05 g.
5. $MgCl_2$	0.05 g.
6. Na_2CO_3	0.1 g.
7. $FePO_4$	0.05N
8. Saccharose	5.0 g.
9. $NaNO_3$	2.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1 liter of water.
(2) Add 8 and 9 to 50.0 cc. of (1).
(3) Make (2) up to 500.0 cc.

Sterilization: Not specified.

Use: To study nitrate reduction by *Clostridium gelatinosum, Bact. Hartlebi*. The authors reported that when more saccharose was added more nitrate was left unchanged.

Variants:

(a) The authors suggested the addition of 2.0 g. of $CaCO_3$ with 5.0 or 17.1 g. sucrose and 2.0 or 4.25 g. $NaNO_3$ per 50.0 cc. solution.
(b) The authors suggested that 5.0 or 17.1 g. sucrose be added with 2.0 or 4.25 g. $NaNO_3$ per 50.0 cc. solution.
(c) The authors added 2.0 g. sucrose and 1.0 or 2.0 g. of NH_4NO_3 to 100.0 cc. of solution.

Reference: Stoklasa and Vitek (1905 p. 198).

344. Czapek's Sucrose Nitrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $NaNO_3$	2.0 g.
3. K_2HPO_4	1.0 g.
4. KCl	0.5 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.01 g.
7. Sucrose	30.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Place in 100.0 cc. portions in flasks. Plugg.
(3) Adjustment of reaction, not given.

Sterilization: Sterilize at 15 pounds for 15 minutes.

Use: Cultivation of soil forms.

Variants: Tanner specified the use of 0.5 g. $MgSO_4 \cdot 7H_2O$.

References: Waksman (1918 p. 479), Tanner (1919 p. 66).

345. Gerlach and Vogel's Cellulose Nitrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $NaNO_3$	5.0 g.
3. Potassium phosphate	0.5 g.
4. $MgSO_4$	0.3 g.
5. NaCl	0.5 g.
6. $NaHCO_3$	0.5 g.
7. $FeSO_4$	some
8. Straw	25.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Distribute in 200.0 cc. lots.
(3) To each flask add 5.0 g. straw.
(4) Adjustment of reaction not given.

Sterilization: Sterilize in the steamer.

Use: Cultivation of albumin formers from soil and stable manure. The author found that if the medium was inoculated with albumin formers alone all the nitrate nitrogen was changed to albuminous nitrogen. When denitrifying bacteria were inoculated with the albumin formers some of the nitrogen was lost.

Reference: Gerlach and Vogel (1901 p. 619).

346. Dubos' Cellulose Sodium Nitrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $NaNO_3$	0.5 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4$	0.5 g.
5. KCl	0.5 g.
6. $FeSO_4$	0.01 g.
7. Filter paper	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Tube in 5 cc. quantities in tubes containing a strip of filter paper. The paper is partially immersed in water.

Sterilization: Not specified.

Use: Isolation of aerobic bacteria decomposing cellulose.

Reference: Dubos (1927 p. 52).

347. Krainsky's Starch Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. Starch (1.0%)	10.0 g.
3. KNO_3 (0.3%)	3.0 g.
4. K_2HPO_4 (0.05%)	0.5 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Enrichment of *Actinomycetes coelicolor, Actinomycetes diastaticus.*

Reference: Krainsky (1914 p. 658).

348. Löhnis' Cellulose Nitrate Solution

Constituents:

1. Water (tap)	1000.0 cc.
2. Cellulose	20.0 g.
3. KNO_3	2.5 g.
4. K_2HPO_4	0.5 g.

Preparation: (1) Dissolve 3, 4 and 20.0 g. of cellulose (filter paper or cotton wool) in 1.

Sterilization: Not specified.

Use: To study denitrification.

Reference: Löhnis (1913 p. 111).

349. Beijerinck and Minkman's Alcohol Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. Ethyl alcohol	5.0 g.
3. KNO_3	10.0 g.
4. K_2HPO_4	0.5 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Inoculate with soil.

(3) Incubate under anaerobic conditions.

Sterilization: Not specified.

Use: To study denitrification by soil forms, and *Bacillus pyocyaneus.* The author reported that the medium became blue green due to *Bacillus pyocyaneus.*

Variants: Tanner used 5.0 cc. of ethyl alcohol and specified the use of tap water.

References: Beijerinck and Minkman (1910 p. 35), Tanner (1919 p. 67).

350. Bokorny's Alcohol Nitrate Solution

Constituents:

1. Water
2. Methyl alcohol
3. KH_2PO_4
4. $MgSO_4$
5. $Ca(NO_3)_2$

Preparation:

(1) Prepare a mineral solution by dissolving 0.05% KH_2PO_4, 0.025% bitter salts and 0.04% $Ca(NO_3)_2$ in water.

(2) Prepare solutions with 0.1% (1) and 1.0% methyl alcohol.

Sterilization: Not specified.

Use: Cultivation of fungus utilizing methyl alcohol as carbon source.

Variants: The author used 10.0, 5.0, 2.0, 0.5, 0.005 or 0.0025% methyl alcohol instead of 1.0%.

Reference: Bokorny (1911 p. 178).

351. Kühne's Glycerol Nitrate Solution
(Proskauer and Beck)

Constituents:

1. Water	1000.0 cc.
2. $Ca(NO_3)_2$	2.0 g.
3. KCl	0.5 g.
4. $MgSO_4$	0.5 g.
5. KH_2PO_4	0.5 g.
6. Glycerol	30.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli. Growth was very scant.

Reference: Proskauer and Beck (1894 p. 131).

352. Weissenberg's Glycerol Nitrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Sodium phosphate (neutral)	2.5 g.
3. Glycerol	40.0 g.
4. NaCl	5.0 g.
5. NH_4NO_3	3.75 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Place 0.75 g. NH_4NO_3 in 175.0 cc. Erlenmeyer flasks.

(3) Fill each flask containing NH_4NO_3 with (1).

Sterilization: Sterilize carefully (method not given).

Use: To study denitrification by *B. pyocyaneus.* The author found that ammonia was produced.

Reference: Weissenberg (1897 p. 284).

353. Lantzsch's Glycerol Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. KNO_3	0.5 to 1.5 g.
3. Glycerin	20.0 g.
4. Malic acid	7.0 g.
5. Na_2HPO_4	0.5 g.
6. NaCl	0.5 g.
7. Na_2CO_3	0.5 g.
8. $MgSO_4$	0.1 g.

Preparation: (1) Dissolve 2, 3, 4 (neutralize acid with soda) 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: To determine nitrate reduction by fluorescens group. Acidify the culture with 5.0% H_2SO_4 and add a strong zinc iodide solution. A blue color indicates nitrate formation or presence. Test for NH_3 with Nessler's reagent.

Reference: Lantzsch (1921 p. 82).

354. Stoklasa's Mannitol Nitrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Mannite	20.0 g.
3. $NaNO_3$	2.0 g.
4. K_2HPO_4	0.5 g.
5. K_2SO_4	0.2 g.
6. $CaCl_2$	0.05 g.
7. $MgCl_2$	0.05 g.
8. $FePO_4$	0.05 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 8 in 1.

Sterilization: Not specified.

Use: To study action of *Azotobacter* and *Radiobacter* on nitrates. Author found that nitrates were reduced.

Reference: Stoklasa (1908 p. 493).

355. Doryland's Acetic Acid Nitrate Solution

Constituents:

1. Distilled water	500.0 cc.
2. HCl dilute	
3. $MgSO_4$	0.5 g.
4. CaO	0.01 g.
5. $Fe_2(SO_4)_3$	0.01 g.
6. $MnSO_4$	0.01 g.
7. HNO_3 (amount not given)	
8. H_2SO_4	
9. CH_3COOH	
10. H_3PO_4	
11. N/0.2578 NaOH	
12. N/0.6205 KOH	

Preparation:

(1) Dilute the HCl so that 1.0 cc. is not quite neutralized by 1.0 cc. silicate solution. Prepared by dissolving 24.0 g. K_2SiO_3 and 8.4 g. Na_2SiO_3 in 500.0 cc. distilled water (phenolphthalein).

(2) Add the following salts to the HCl:

$MgSO_4$	0.5 g.
CaO	0.01 g.
$Fe_2(SO_4)_3$	0.01 g.
$MnSO_4$	0.01 g.
HNO_3 (amount not given)	

(3) Standardize (2) so 1.0 cc. equivalent to 1.0 cc. silicate solution, using methyl orange as indicator.

(4) Standardize a solution of H_2SO_4 in the same way as HCl omitting the salts.

(5) Standardize H_3PO_4 and CH_3COOH solution in the same way as HCl omitting the salts and using phenolphthalein as indicator.

(6) Mix the acids in the following ratio:

HCl	153.5 cc.
CH_3COOH	153.5 cc.
H_2SO_4	77.0 cc.
H_3PO_4	116.0 cc.

(7) Mix equal quantities of N/0.6205 KOH and N/0.2578 NaOH solutions.

(8) 1.0 cc. of (7) should neutralize 1.0 cc.

(6) using phenolphthalein as indicator.

(9) Acid and base solutions are to be drawn into separately plugged burettes and allowed to stand several hours to sterilize.

(10) Mix equal quantities of (6) and (7) when ready for use.

Sterilization: Method not specified.

Use: As a general synthetic medium.

Reference: Doryland (1916 pp. 146–148).

356. Giltay and Aberson's Citrate Nitrate Solution (Murray)

Constituents:

1. Water	1000.0 cc.
2. KNO_3	2.0 g.
3. $MgSO_4$	2.0 g.
4. Citric acid	5.0 g.
5. K_2HPO_4	2.0 g.
6. $CaCl_2$	0.20 g.
7. Na_2CO_3	4.25 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: To study denitrification under aerobic and anaerobic conditions.

Variants:

(a) Fred used 0.2 g. K_2HPO_4 instead of 2.0 g. K_2HPO_4.

(b) Fred used 2.0 g. $NaNO_3$ instead of 2.0 g. KNO_3.

(c) Christensen omitted the Na_2CO_3, used 3.0 g. of $NaNO_3$ instead of KNO_3, used 0.5 g. sodium citrate instead of citric acid and added a trace of iron chloride.

(d) Harvey and Percival omitted the Na_2CO_3, added a trace of $FeCl_3$ and used 2.0 g. of Na_2NO_3 instead of 2.0 g. KNO_3.

References: Murray (1916 p. 611), Fred (1911–12 p. 422), Christensen (1913 p. 421), Harvey (1921–22 p. 108), Percival (1920 p. 168).

357. Percival's Tartrate Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. Calcium tartrate	20.0 g.
3. KNO_3	20.0 g.
4. K_2HPO_4	0.5 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Method not specified.

Use: To study denitrification.

Reference: Percival (1920 p. 167).

358. Gottheil's Nitrate Solution No. VII

Constituents:

1. Distilled water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.3 g.
5. NaCl	0.1 g.
6. Iron	trace
7. KNO_3	10.0 g.
8. Sucrose	5.0 g.
9. Glycerol	10.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

Sterilization: Not specified.

Use: General culture medium for organisms found in the soil, on roots and on rhizomes.

Reference: Gottheil (1901 p. 432).

359. Burri and Stutzer's Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. KNO_3	2.0 g.
3. Glucose	2.0 g.
4. $MgSO_4$	2.0 g.
5. Citric acid	5.0 g.
6. Potassium phosphate	2.0 g.
7. $CaCl_2$	0.2 g.
8. $FeCl_3$	2 drops

Preparation:

(1) Dissolve 2 and 3 in part of 1.

(2) Dissolve 4, 5, 6, 7 and 8 in the remainder of 1.

(3) Boil (2) over a flame and neutralize with soda.

(4) Mix (1) and (3) and make up to 1 liter.

Sterilization: Sterilize in the steamer.

Use: To study nitrate reduction by *B. denitrificans*.

Variants:

(a) Jensen used 2.0 g. $NaNO_3$ instead of 2.0 g. KNO_3, specified the use of K_2HPO_4 and omitted the $FeCl_3$.

(b) v. Bazarewski used either 2.0 g. of $NaNO_3$ or KNO_3 and specified the use of KH_2PO_4.

Reference: Burri and Stutzer (1895 p. 393), Jensen (1897 p. 623), v. Bazarewski (1905 p. 4).

360. Kuntze's Nitrate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Salt peter	2.0 g.
3. Glucose	2.0 g.
4. $MgSO_4$	2.0 g.
5. Citric acid	5.0 g.
6. Potassium sulphate	2.0 g.
7. $CaCl_2$	0.2 g.
8. Iron chloride	2 drops

Preparation:

(1) Dissolve 2 and 3 in part of 1.

(2) Dissolve 4, 5, 6, 7 and 8 in the remainder of 1.

(3) During the heating of (2) neutralize with soda.

(4) Mix (3) and (1).

(5) Distribute into 100.0 cc. lots in combustion flasks.

(6) Inoculate with 5.0 g. manure.

Sterilization: Method not given.

Use: To study morphology and physiology of denitrifying organisms, *Bacillus denitrificans agilis (Ampola and Garino)* and *Bacillus oxalaticus (Zopf).*

Variants: Giltner gives the following solution:

1. Distilled water	1000.0 g.
2. KH_2PO_4	2.0 g.
3. $MgSO_4$	2.0 g.
4. KNO_3	1.0 g.
5. $CaCl_2$	0.2 g.
6. Fe_2Cl_6 solution	2.0 drops
7. Citric acid	5.0 g.
8. Glucose	10.0 g.

References: Kuntze (1904 p. 2), Giltner (1921 p. 376).

361. Giltay and Aberson's Nitrate Solution (Stoklasa and Vitek)

Constituents:

1. Water	1000.0 cc.
2. $NaNO_3$	2.0 g.
3. K_2HPO_4	2.0 g.
4. $CaCl_2$	0.2 g.
5. Glucose	2.0 g.
6. Citric acid	5.0 g.
7. Iron chloride	few drops

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Neutralize 5.0 g. citric acid with soda.

(3) Add (2) and several drops of iron chloride to (1).

Sterilization: Not specified.

Use: To study nitrate reduction.

Reference: Stoklasa and Vitek (1905 p. 104).

362. Giltay's Sucrose Nitrate Solution (Fred)

Constituents:

1. Distilled water	1000.0 cc.
2. $NaNO_3$	2.0 g.
3. $MgSO_4$	2.0 g.
4. K_2HPO_4	2.0 g.
5. $CaCl_2$	0.2 g.
6. Citric acid	5.0 g.
7. Na_2CO_3	4.25 g.
8. Sucrose	2.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: To study nitrate reduction by *B. fluorescens liquefaciens*, *B. pyocyaneus*, *B. Hartlebi* (H. Jensen), *B. denitrificans*.

Reference: Fred (1911–12 p. 422).

363. Behren's Cellulose Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. NH_4NO_3	10.0 g.
3. Sucrose	2.0 g.
4. Potassium phosphate	5.0 g.
5. $MgSO_4$	1.0 g.
6. Lactic acid	2.0 g.
7. Swedish filter paper	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Filter.

(3) Distribute in 100.0 cc. lots.

(4) Add pieces of purified Swedish filter paper to each flask.

Sterilization: Not specified.

Use: To study utilization of cellulose by *Botrylis vulgaris*, *Pencillium glaucum* and *Penicillium luteum.* The author reported that *Botrylis vulgaris* dissolved the cellulose.

Reference: Behrens (1898 p. 549).

364. Maassen's Malic Acid Nitrate Solution (Stoklasa and Vitek)

Constituents:

1. Water	1000.0 cc.
2. NaCl	0.5 g.
3. Na_2CO_3	0.5 g.
4. $MgSO_4$	0.1 g.
5. Malic acid	7.0 g.

6. Glycerol	20.0 g.
7. $NaNO_3$	2.5 g.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Neutralize 7.0 g. malic acid with Na_2CO_3.
(3) Add (2), 6 and 7 to (1).

Sterilization: Not specified.

Use: To study nitrate reduction.

Reference: Stoklasa and Vitek (1905 p. 104).

365. Maassen's Stearate Nitrate Solution (Vierling)

Constituents:

1. Water	1000.0 cc.
2. Glycerin	20.0 g.
3. KNO_3	2.5 g.
4. Potassium stearate	1.0 g.
5. $MgSO_4$	1.0 g.
6. K_2HPO_4	0.5 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study the reduction of nitrate by Mycobacteria. Author reported that little or no ammonia was produced.

Reference: Vierling (1920 p. 202).

SUBGROUP I-C. SECTION 5

Liquid media or basal solutions with constituents of known chemical composition; organic nitrogen present as amino acids; other forms of organic nitrogen may also be present.

A_1.* Only one amino acid present.
B_1.† Incomplete or basal solutions, used with the addition of a variety of other nutrients.
C_1. Amino nitrogen supplied as glycocoll.
  Went's Basal Glycocoll Solution.... 366
  Wherry's Basal Glycocoll Solution... 367
  Waksman's Basal Glycerol Glycocoll Solution.... 368
C_2. Amino nitrogen supplied as alanine.
  Went's Basal Alanin Solution....... 369
C_3. Amino nitrogen supplied as tyrosine.
  Went's Basal Tyrosine Solution..... 370
C_4. Amino nitrogen supplied as leucine.
  Waksman's Basal Glycerol Leucine Solution.... 371
  Went's Basal Leucine Solution...... 372
C_5. Amino nitrogen supplied as aspartic acid or asparagin.
D_1. Containing no other added organic carbon.
  van Delden's Basal Asparagin Salt Solution.... 373
  Uschinsky's Basal Asparagin Solution (Kendall, Day and Walker)....... 374
  Kita's Basal Asparagin Solution..... 375
  Proskauer and Beck's Basal Asparagin Solution.... 376
  Hefferan's Basal Asparagin Solution. 377
  Zikes' Basal Asparagin Solution..... 378
  Miehe's Basal Asparagin Solution... 379
  Gottheil's Basal Asparagin Solution. 380
  Went's Basal Asparagin Solution.... 381
  Sullivan's Basal Asparagin Solution. 382
D_2. Containing additional organic carbon.
E_1. Only one type of organic carbon added.
F_1. Carbohydrates, only, added.
  Munter's Basal Glucose Asparagin Solution.... 383
  Ono's Basal Sucrose Asparagin Solution.... 384
  Bokorny's Basal Asparagin Solution. 385
  Higgins' Basal Carbohydrate Asparagin Solution.... 386
F_2. Alcohols only added.
  Peklo's Basal Asparagin Solution... 387
  Sullivan's Basal Glycerol Asparagin Solution.... 388
  Waksman's Basal Glycerol Asparagin Solution.... 389
F_3. Acids or their salts, only, added.
  Tanner's Basal Lactate Asparagin Solution.... 390
  Hiss' Basal Litmus Asparagin Solution.... 391
  Harvey's Basal Lactate Asparagin Solution.... 392
  Capaldi and Proskauer's Basal Citrate Asparagin Solution.... 393
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  Maassen's Basal Malic Acid Asparagin Solution (Smith).... 395
E_2. More than one type of organic carbon added.
  Lockemann's Basal Citrate Glycerol Solution.... 396
  Mendel's Basal Citrate Glycerol Solution.... 397
B_2. Complete media.

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* See page 104 for A_2.

† See bottom of next column for B_2.

C_1.* Organic nitrogen supplied only as amino acids or their salts.
D_1. Amino nitrogen supplied as glycocoll.
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F_1.* Only one type of organic carbon added.
G_1. Additional carbon supplied as carbohydrates.
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*See next page for C_2.

†See next column for E_2.

*See next page for F_2.

G_2. Additional carbon supplied as organic acids or their salts.
H_1. Acetic acid or its salts added.
  Ellrodt's Acetate Asparagin Solution.... 441a
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  Voges' Lactate Asparaginate Solution.... 441b
  Frankel and Voges' Salt Asparagin Solution (Besson).... 441c
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I_2. Ammonium salts not added.
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H_3. Citric acid or its salts added.
  Kuntze's Citrate Asparagin Solution. 450
  Giltay and Aberson's Citrate Asparagin Solution (Tanner).... 451
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H_4. Malic acid or its salts added.
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F_2. More than one type of additional organic carbon employed.
G_1. Carbohydrates and alcohols, only, added.
  Zikes' Alcohol Asparagin Solution.. 455
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  Muller, Thurgan and Osterwalder's Malate Asparagin Solution.... 459
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  Lockemann's Citrate Glycerol Asparagin Solution (Löwenstein).... 467
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C_2. Organic nitrogen present in addition to amino nitrogen.
D_1. Amino nitrogen present as glycocoll.
  Hadley's Urea Glycocoll Solution... 469
D_2. Amino nitrogen present as aspartic acid or asparagin.
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A_2. More than one amino acid present.
B_1. Incomplete or basal solutions employed with the addition of other materials.
  Davis and Ferry's Basal Cystine Tryptophane Solution.... 474
B_2. Complete media.
C_1. Containing glycocoll.
  Berthelot's Amino Acid Solution.... 475
  Armand-Delille, et al., Glycocoll Arginine Solution (Synthetic Medium 164).... 476
  Armand-Delille, et al., Synthetic Solution 104.... 477
  Armand-Delille, et al., Glycocoll Aspartic Acid Solution (Synthetic Medium 118).... 478
C_2. Not containing glycocoll.
  Zipfel's Tryptophane Asparagin Solution.... 479

366. Went's Basal Glycocoll Solution

Constituents:

1. Water.... 1000.0 cc.
2. Glycocoll (0.66%).... 6.6 g.

Preparation:

(1) Prepare 5.0% solutions of one of the materials listed in added nutrients.
(2) Flask in 25.0 cc. quantities.
(3) Prepare a 0.66% solution of glycocoll.
(4) Add 25.0 cc. of (3) to each flask of (2).

Sterilization: Method not given.

Use: To study nutrients for *Monilia sitophila* (*Mont.*) *Sacc.* The materials ranked as follows as suitable additional carbon sources: maltose, glucose, lactose, sucrose and glycerol.

Added nutrients: The author used the following additional carbon sources:

maltose
lactose
sucrose
glucose
glycerol

Variants: The author used the basic solution without additional materials.

Reference: Went (1901 p. 593).

367 Wherry's Basal Glycocoll Solution

Constituents:

1. Redistilled water	1000.0 cc.
2. NaCl	1.0 g.
3. KCl	1.0 g.
4. $CaCl_2$	1.0 g.
5. $MgSO_4$	1.0 g.
6. Glycocoll	2.0 g.
7. $(NH_4)_2HPO_4$	2.0 g.

(Salts to be Kahlbaum's C.P.)

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Add one of the added nutrients.
(3) Adjustment of reaction not given.

Sterilization: Sterilize in autoclave, time not given.

Use: To study morphology of *B. tuberculosis.* Growth appeared as very thin scattered pellicles composed of non-acid-proof coccoid bodies and thin segmented rods.

Added nutrients: The author added one of the following:

$(NH_4)_2HPO_4$	2.0 g.
$(NH_4)_2HPO_4$ $(NH_4)_2SO_4$	2.0 g. 2.0 g.
KH_2PO_4 NH_4Cl	2.0 g. 2.0 g.
KH_2PO_4 $(NH_4)_2SO_4$	2.0 g. 2.0 g.

Reference: Wherry (1913 p. 152).

368. Waksman's Basal Glycerol Glycocoll Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol	30.0 g.
3. K_2HPO_4	1.0 g.
4. KCl	0.5 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.01 g.
7. Glycocoll	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 and 2.0 g. of one of the added nutrients in 1.
(2) Tube in 10–12 cc. lots.
(3) Adjustment of reaction not specified.

Sterilization: Sterilize at 15 pounds for 15 minutes.

Use: To study metabolism of actinomycetes.

Added nutrients: The author added 2.0 g. of one of the following:

$NaNO_3$
$NaNO_2$
$(NH_4)_2SO_4$
$(NH_4)_2CO_3$

Reference: Waksman (1920 p. 3).

369. Went's Basal Alanin Solution

Constituents:

1. Water	1000.0 cc.
2. Alanin (0.66%)	6.6 g.

Preparation:

(1) Prepare 5.0% solutions of one of the materials listed in added nutrients.
(2) Flask in 25.0 cc. quantities.
(3) Prepare a 0.66% solution of alanin.
(4) Add 25.0 cc. of (3) to each flask of (2).

Sterilization: Method not given.

Use: To study the nutrients for *Monilia sitophila* (*Mont.*) *Sacc.* The materials ranked as follows as suitable carbon sources with alanin as a nitrogen source: glycerol, sucrose, maltose, lactose and glucose.

Added nutrients: The author used the following additional carbon sources:

maltose
lactose
sucrose
glucose
glycerol

Variants: The author used the basal solution without additional materials.

Reference: Went (1901 p. 593).

370. Went's Basal Tyrosine Solution

Constituents:

1. Water	1000.0 cc.
2. Tyrosine (0.66%)	6.6 g.

Preparation:

(1) Prepare 5.0% solutions of one of the materials listed in added nutrients.
(2) Flask in 25.0 cc. quantities.
(3) Prepare a 0.66% tyrosine solution.
(4) Add 25.0 cc. of (3) to each flask of (2).

Sterilization: Method not given.

Use: To study nutrients for *Monilia sitophila* (*Mont.*) *Sacc.* The materials ranked as follows as suitable carbon sources with tyrosine as a nitrogen source: maltose, glucose, sucrose, lactose, glycerol.

Added nutrients: The author used the following additional carbon sources:

maltose
lactose
sucrose
glucose
glycerol

Variants: The author used the basic solution without additional materials.

Reference: Went (1901 p. 593).

371. Waksman's Basal Glycerol Leucine Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol	30.0 g.
3. K_2HPO_4	1.0 g.
4. KCl	0.5 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.01 g.
7. Leucine	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 2.0 g. of one of the added nutrients in 1.
(2) Tube in 10–12 cc. lots.
(3) Adjustment of reaction not specified.

Sterilization: Sterilize at 15 pounds for 15 minutes.

Use: To study metabolism of actinomycetes.

Added nutrients: The author added 2.0 g. of one of the following:

$NaNO_3$
$NaNO_2$
$(NH_4)_2SO_4$
$(NH_4)_2CO_3$

Reference: Waksman (1920 p. 3).

372. Went's Basal Leucine Solution

Constituents:

1. Water	1000.0 cc.
2. Leucine (0.66%)	6.6 g.

Preparation:

(1) Prepare 5.0% solutions of one of the materials listed in added nutrients.
(2) Flask in 25.0 cc. quantities.
(3) Prepare a 0.66% solution of leucine.
(4) Add 25.0 cc. of (3) to each flask of (2).

Sterilization: Method not given.

Use: To study the nutrients for *Monilia sitophila* (*Mont.*) *Sacc.* The materials ranked as follows as suitable carbon sources with leucine as nitrogen source: glucose and maltose, sucrose, glycerol, lactose.

Added nutrients: The author used the following additional carbon sources:

maltose
lactose
sucrose
glucose
glycerol

Variants: The author used the basic solution without additional materials.

Reference: Went (1901 p. 593).

373. van Delden's Basal Asparagin Salt Solution

Constituents:

1. Water, tap	1000.0 cc.
2. K_2HPO_4	0.5 g.
3. Asparagin	5.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Additional nutrients are added as indicated below.

Sterilization: Not specified.

Use: To study sulphate reduction by *Microspira desulfuricans*.

Added nutrients and variants: The author employed the following solutions:

(a) Added 2.0 g. $MgSO_4 \cdot 7H_2O$.
(b) 5.0 g. potassium succinate, 5.0 g. gypsum and 1.0 g. of asparagin.
(c) 5.0 g. potassium citrate, 5.0 g. gypsum and 1.0 g. of asparagin.
(d) Specified the use of sea water in the preparation of the basic solution, used 1.0 g. of asparagin and added 5.0 g. of sodium lactate or 2.0 g. glucose.
(e) 1.0 g. asparagin in the basic solution and added 30.0 g. NaCl, and 8.0 g. $MgSO_4 \cdot 7H_2O$. To this solution he added 0.0, 5.0 or 10.0 g. of sodium lactate.
(f) Added 30.0 g. NaCl and 12.0 g. of gypsum to the basic solution.

(g) Added 10.0 g. glucose, 30.0 g. NaCl and 8.0 g. $MgSO_4 \cdot 7H_2O$ to the basic solution.

(h) Used 2.5 g. asparagin in the basic solution and added 30.0 g. NaCl, 8.0 g. $MgSO_4 \cdot 7H_2O$ and 5.0 g. sodium lactate.

(i) Used 1.0 g. asparagin in the basic solution, added 8.0 g. $MgSO_4 \cdot 7H_2O$, 30.0 g. NaCl and 5.0 g. of sodium lactate or potassium succinate.

(j) Used 1.0 g. of asparagin in the basic solution and added 5.0 g. sodium lactate, 75.0 g. NaCl and 8.0 g. $MgSO_4 \cdot 7H_2O$.

Reference: van Delden (1903–04 p. 85, 105).

374. Uschinsky's Basal Asparagin Solution (Kendall, Day and Walker)

Constituents:

1. Distilled water	1000.0 cc.
2. Asparagin	4.0 g.
3. Na_2HPO_4	2.0 g.
4. NaCl	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and one of the materials listed under added nutrients in 1.

(2) Distribute in 100.0 cc. lots.

(3) Adjustment of reaction not given.

Sterilization: Method not given.

Use: To study metabolism by the tubercle bacilli. Authors used alizarin, neutral red and phenolphthalein to study changes in reaction; Ziehl-Neelsen stain for staining.

Added nutrients: The following organic materials were added:

glucose	10.0 g.
mannitol	10.0 g.
glycerol	30.0 g.

The following materials were added to study lipase production: different esters were incubated for 24 hours with clear bacteria free culture broth, and amount of acid produced, measured in terms of n/50 NaOH, determined lipase production.

ethyl alcohol and $(NH_4)_2HPO_4$ amounts not given.

glycerol and $(NH_4)_2HPO_4$ amounts not given.

glucose and $(NH_4)_2HPO_4$ amounts not given.

References: Kendall, Day and Walker (1914 p. 428), Kendall, Walker and Day (1914 p. 355).

375. Kita's Basal Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4$	2.5 g.
3. KH_2PO_4	5.0 g.
4. $FeCl_4$ solution	drops
5. Asparagin	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add 5.0% of any carbohydrate.

Sterilization: Not specified.

Use: Cultivation of Japanese molds, *Aspergillus Okazaki*, *Aspergillus candidus*, *Aspergillus albus*, *Aspergillus tamarii*, *Pseudorhizopus*, *Aspergillus glaucum.*

Added nutrients: Add 5.0% of any carbohydrate.

Variants:

(a) Buromsky used the following solution to study effect of organic acids on yeast, *Saccharomyces ellipsoid*, *Saccharomyces pastorianus.*

1. Water	1000.0 g.
2. $MgSO_4$	0.5 g.
3. KH_2PO_4	1.0 g.
4. Asparagin	5.0 g.

The solution was prepared by

(1) Dissolve 2, 3 and 4 in 1.

(2) Distribute in 100.0 cc. lots in 500.0 cc. Erlenmeyer flasks.

(3) Add one of the listed materials below as additional carbon source.

(4) Sterilize on 3 successive days in a Koch steamer, 30 minutes the first day, and 20 minutes on each the 2nd and 3rd day, (a slight turbidity develops when glycerin or mannite is added).

The following nutrients were added:

quinic acid	0.5%
citric acid	1.0%
tartaric acid	1.0%
succinic acid	1.0%
glycerol	1.0%
mannitol	1.0%

(b) von Bronsart used 0.25% KH_2PO_4 and 5.0% asparagin instead of 0.1% KH_2PO_4 and 0.5% asparagin. He added one of the following carbon sources (amount not specified).

starch
dextrin
sucrose
levulose
glucose

He used the solution for the cultivation of *Xylaria*, *Xylaria hypoxylon*.

References: Kita (1913 p. 434), Buromsky (1914 p. 532), von Bronsart (1919 p. 59).

376. Proskauer and Beck's Basal Asparagin Solution

Constituents:
1. Water .... 1000.0 cc.
2. Potassium phosphate (0.5%) .... 5.0 g.
3. $MgSO_4$ (0.25%) .... 2.5 g.
4. Asparagin (0.5%) .... 5.0 g.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Add one of the combinations listed under added nutrients.

Sterilization: Not specified.

Use: To study the constituents essential for the growth of tubercle bacilli.

Added nutrients: The authors added one of the following combinations:
(a) citric acid 0.075%
  glycerol 4.0%
  glucose 1.0%
  K_2SO_4 .... 0.25%
  NaCl 0.15%
(b) citric acid 0.75%
  glycerol 4.0%
  K_2SO_4 .... 0.25%
  NaCl 0.15%
(c) Glycerol 4.0%
  glucose .... 1.0 or 0.0%
  K_2SO_4 .... 0.25%
  NaCl 0.15%
(d) Glycerol 1.5%
  K_2SO_4 .... 0.25%
  NaCl 0.15%
(e) Glycerol 1.0%
  K_2SO_4 .... 0.25%
  NaCl 0.15%
(f) Glycerol 1.0%
  One of the following:
  citric acid 1.0%
  lactic acid 0.1%
  tartaric acid 0.75%
  malic acid 0.75%
  succinic acid 0.75%
  aspartic acid 0.75%
  alanin 0.75%
(g) Glycerol 1.0%
  citric acid 0.1%
  alanin 0.1%
(h) citric acid 1.5%
  One of the following:
  alanin 0.1%
  fumaric acid 0.075%
  aconitic acid 0.075%
  citraconic acid 0.075%
  itaconic acid 0.075%
(i) citric acid 1.0%
  glyceric acid 1.0%
  or
  Biuret 0.075%

Variants:
(a) The authors used 0.3% potassium phosphate and added 0.25% magnesium citrate and 1.5% citric acid.
(b) The authors used 0.06% $MgSO_4$ and added 0.25% magnesium citrate and 1.5% citric acid.
(c) The authors used 0.1, 0.2, 0.3, 0.4, 0.5 or 0.6% asparagin without additions.

Reference: Proskauer and Beck (1894 p. 139).

377. Hefferan's Basal Asparagin Solution

Constituents:
1. Redistilled water .... 1000.0 cc.
2. Asparagin .... 2.0 g.

Preparation:
(1) Dissolve 2 in redistilled water.
(2) Reaction is neutral to phenolphthalein.
(3) Distribute in Jena flasks that have been cleaned in acid and rinsed repeatedly in distilled water.
(4) Add one of the combinations given below.

Sterilization: Not specified.

Use: To study pigment production by red pigment forming bacteria, *B. prodigiosus*, *B. ruberbalticus*, *B. kiliensis*, *B. rubermiquel*, *B. rutilus*, *B. amyloruber*. Pigment production generally poor.

Added nutrients: One of the following materials or combinations may be added:
(a) 1.0 g. $MgSO_4$ and 1.0 g. K_2HPO_4.
(b) 1.0 g. K_2HPO_4.
(c) 1.0 g. $MgSO_4$.
(d) 1.0 g. $MgSO_4$, 1.0 g. K_2HPO_4 and 20.0 g. glycerol.

(e) 1.0 g. $MgSO_4$, 1.0 g. K_2HPO_4 and 10.0 g. glucose.
(f) 1.0 g. $MgSO_4$, 1.0 g. K_2HPO_4 and 10.0 g. lactose.
(g) 1.0 g. $MgSO_4$, 1.0 g. K_2HPO_4 and 10.0 g. sucrose.

Reference: Hefferan (1903–4 p. 522).

378. Zikes Basal Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin	2.5 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4$	0.3 g.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Add 10.0% of one of the carbon sources listed under added nutrients.

Sterilization: Not specified.

Use: Volutin production by yeast, *Oidium lactis*.

Added nutrients: The author used 10.0% of one of the following carbon sources:

glucose
fructose
galactose
maltose
sucrose
lactose
raffinose
dextrin
inulin

Reference: Zikes (1922 p. 34).

379. Miehe's Basal Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4 (0.1%)	1.0 g.
3. $MgSO_4$ (0.02%)	0.2 g.
4. $CaCl_2$ (0.01%)	0.1 g.
5. Asparagin (0.5%)	5.0 g.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Add 4.0% of one of the added nutrients.

Sterilization: Not specified.

Use: To study carbon sources suitable for tubercle bacilli.

Added variants: The following materials were added:

glycerol	4.0%
dextrin	4.0%
glucose	4.0%
maltose	4.0%
xylose	4.0%

Reference: Miehe (1908–9 p. 137).

380. Gottheil's Basal Asparagin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.3 g.
5. NaCl	0.1 g.
6. Iron	trace
7. Asparagin	10.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Add one of the added nutrients in the amount indicated.

Sterilization: Not specified.

Use: Cultivation of organisms found in the soil, on roots and on rhizomes.

Added nutrients: One of the following combinations were added:

(a)	glycerol	10.0 g.
	sucrose	5.0 g.
(b)	sucrose	5.0 g.
(c)	galactose	3.0 g.
	lactose	3.0 g.
(d)	galactose	3.0 g.
	glycerol	3.0 g.

Reference: Gottheil (1901 p. 432).

381. Went's Basal Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin (0.66%)	6.6 g.

Preparation:
(1) Prepare 5.0% solutions of one of the materials listed in added nutrients.
(2) Flask in 25.0 cc. quantities.
(3) Prepare a 0.66% solution of asparagin.
(4) Add 25.0 cc. of (3) to each flask of (2).

Sterilization: Method not given.

Use: To study the nutrients for *Monilia sitophila* (*Mont.*) *Sacc.* The materials ranked as follows as suitable carbon sources, with nitrogen supplied as asparagin: sucrose, glycerol, glucose, maltose and lactose.

Added nutrients: The author used the following additional carbon sources:

maltose
lactose
sucrose
glucose
glycerol

Variants: The author used the basic solution without additional materials.

Reference: Went (1901 p. 593).

382. Sullivan's Basal Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin	10.0 g.

Preparation:

(1) Dissolve 2 in 1.

(2) Add one of the materials listed under added nutrients.

Sterilization: Not specified.

Use: To study pigment formation. The author reported that the blue-green pyocyanin was formed whenever there was present a suitable combination of carbon, hydrogen, oxygen, and nitrogen and some salts to aid synthesis. Its production was independent of either a phosphate or a sulphate.

Added nutrients:

(a)	$MgSO_4$	2.0 g.
	K_2HPO_4	1.0 g.
	Na_2S	0.2 g.
(b)	$MgSO_4$	0.2 g.
(c)	K_2HPO_4	1.0 g.
	Na_2SO_4	1.0 g.
(d)	$MgSO_4$	0.2 g.
	K_2HPO_4	1.0 g.
(e)	$MgSO_4$	0.01 to 1.0 g.
	K_2HPO_4	1.0 g.
(f)	$MgSO_4$	1.0 to 16.0 g.
	peptone	10.0 g.
(g)	$MgSO_4$	0.2 g.
	K_2HPO_4	0.01 to 5.0 g.
(h)	$MgSO_4$	0.2 g.
	K_2HPO_4	1.0 g.
	peptone	5.0 g.

Variants: The author used 5.0 g. asparagin and added 0.2 g. $MgSO_4$, 1.0 g. K_2HPO_4, and 4.0 g. ammonium lactate.

Reference: Sullivan (1905–06 pp. 127–140).

383. Münter's Basal Glucose Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl	0.5 g.
3. $MgSO_4$	0.5 g.
4. $CaCl_2$	0.1 g.
5. K_2HPO_4	1.5 g.
6. NH_4NO_3	1.0 g.
7. Asparagin	0.5 g.
8. Dextrose	9.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

(2) Neutralize with Na_2CO_3.

(3) Distribute in 50.0 cc. lots in small Erlenmeyer flasks.

(4) Add one of the added nutrients.

Sterilization: Method not given.

Use: Cultivation of *Actinomyces odorifer*, *Act. chromogenes*, *Act. albus I and II*, *Act. S. a, b, and c*. The author reported that the actinomycetes preferred a neutral medium. They tolerated a very small amount of acid however. No growth in highly acid solutions.

Added nutrients: Add one of the following to each flask:

acetic acid 0.01% or 0.1%.

succinic acid 0.004%, 0.04% or 0.1%.

malic acid 0.004%, 0.01% or 0.1%.

aspartic acid 0.01%, 0.05%, 0.1%, 0.2% or 0.4%.

Variants: The author used the basic solution without any additions.

Reference: Münter (1913 p. 378).

384. Ono's Basal Sucrose Asparagin Solution

Constituents:

1. Water	1000.0 g.
2. Sucrose	85.0 g.
3. Asparagin	16.0 g.
4. $MgSO_4$	3.0 g.
5. KH_2PO_4	5.0 g.
6. $FeSO_4$	trace

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Distribute in 30.0 cc. lots.

Sterilization: Sterilize in the steamer.

Use: To study the effect of small amounts of chemicals on the growth of *Aspergillus niger*. The author reported that small amounts of $CuSO_4$ tended to increase growth while concentrations higher than 1/250 molar $CuSO_4$ tended to hinder growth.

Variants and added nutrients:

(a) The author added $CuSO_4$ solution so that the medium was from 1/32,000 to 1/250 molar $CuSO_4$.

(b) Henneberg used 0.3% asparagin, 0.2% $MgSO_4$, 5.0% sucrose and omitted the $FeSO_4$. To this solution he added the following materials:

NaCl	0.1%
$CaCl_2$	0.1%
K_2SO_4	0.1%

Iron sulphate	0.01%
Calcium lactate	1.0%
gypsum	0.1%
K_2CO_3	0.5%
soda	0.1 to 0.6%
$MgCO_3$	0.1 to 1.0%

(c) Henneberg used 3.0 to 6.0% asparagin, 10.0 to 15.0% sucrose, 0.2% $MgSO_4$ and omitted the $FeSO_4$ from the basic solution. To this solution he added one of the following:

dextrin	1.0%
ammonium tartrate	0.3%
peptone	0.5%
potassium phosphate	0.5%
K_2HPO_4	0.5%
peptone $CaCl_2$	0.5% 0.1%
$CaCl_2$ Chalk	0.1%

(d) Henneberg used 0.3% asparagin, 15.0% sucrose, 0.2% $MgSO_4$ and specified the use of either KH_2PO_4 or K_2HPO_4. To this solution he suggested that one of the following might be added:

gypsum	2.0%
potassium phosphate	2.0%
$CaCl_2$	0.6%
potassium lactate	2.0%
K_2CO_3	2.0%
soda	0.6%
K_2SO_4	0.6%

(e) Bokorny used a medium containing 0.5% asparagin, 0.03% $MgSO_4$, a trace of $CaCl_2$, 10.0% sucrose and added one of the following:

K_2HPO_4 0.1, 0.5, 1.0, 2.0 or 4.0%
KH_2PO_4 0.1, 0.5, 1.0, 2.0 or 4.0%
Na_2HPO_4 0.1, 0.5, 1.0, 2.0 or 4.0%
KH_2PO_4 0.1% with NaCl 0.5, 1.0, 2.0 or 4.0%
KH_2PO_4 0.1% with KI 0.01, 0.05, 0.1 or 0.5%
KH_2PO_4 0.1% with KBr 0.01, 0.05, 0.1 or 0.5%
KH_2PO_4 0.1% with Na_2CO_3 0.5 or 1.0%
KH_2PO_4 0.1% with NaF 0.05, 0.1 or 0.5%
KH_2PO_4 0.1% with $Na_2S_2O_3$ 0.5 or 1.0%

He reported that 4.0% K_2HPO_4, KH_2PO_4 or Na_2HPO_4 did not inhibit the growth of yeast, 2 and 4.0% NaCl inhibited growth. Generally KI and KBr were toxic for yeast. Na_2CO_3 inhibited growth. NaF inhibited growth of yeast. A heavier growth of yeast appeared on medium containing only 0.5% $Na_2S_2O_3$.

(f) Bokorny used a medium containing 1.0% sucrose, 0.1% asparagin, 0.1% KH_2PO_4, 0.05% $MgSO_4$ and a trace of iron chloride. He reported that yeast would grow more luxuriantly if the medium contained 0.05% $CaCl_2$. Calcium was thought to be a necessity for growth. Growth occurred in the solution without calcium due to the probable presence of calcium in the sucrose or asparagin as an impurity.

(g) Bokorny reported that spirogyra grew much more luxuriant and more characteristic in the following medium when 0.05% $CaCl_2$ was added:

1.	Water	1000.0 g.
2.	Sucrose	10.0 g.
3.	Asparagin	2.5 g.
4.	KH_2PO_4	0.5 g.
5.	$MgSO_4$	0.5 g.
6.	$CaCl_2$	0.0 or 0.5 g.

(h) Zikes used the following solution to study perithecium formation by *Aspergillus oryzae:*

1.	Water	1000.0 g.
2.	Asparagin	10.0 g.
3.	K_2HPO_4	5.0 g.
4.	$MgSO_4$	2.5 g.
5.	Sucrose	75.0 g.

(i) Zikes used the following solution to study volutin production by a number of wine and other yeasts:

1.	Water, distilled	1000.0 cc.
2.	Sucrose	100.0 g.
3.	Asparagin	2.5 g.
4.	K_2HPO_4	1.0 g.
5.	$MgSO_4$	0.3 g.

$pH = 5.0706, CH = 8.50 \times 10^{-6}$

References: Ono (1902 p. 155), Henneberg (1907 pp. 40–45), (1909 pp. 104, 105), Bokorny (1912 pp. 119, 120, 135, 138, 144, 147), Zikes (1922 pp. 29, 340).

385. Bokorny's Basal Asparagin Solution

Constituents:

1.	Water	1000.0 cc.
2.	Sucrose	100.0 g.
3.	Asparagin (0.5%)	5.0 g.
4.	KH_2PO_4 (0.1%)	1.0 g.

5. $MgSO_4$ (0.03%)	0.3 g.
6. $CaCl_2$	trace

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Add one of the salts given in added nutrients.

Sterilization: Not specified.

Use: To study the effect of metallic salts on development of yeast.

Added nutrients: The following salts were added:

$CaSO_4$ 0.1 or 0.02 g.
$MnSO_4$ 1.0, 3.0 or 5.0 g.
$KMnO_4$ 0.1, 0.05 or 0.01 g.
$NiSO_4$ 0.02, 0.03 or 0.01 g.
K_2CrO_4 (red 1/3 neutral) 0.1, 0.05 or 0.5 g.
K_2CrO_4 (yellow) 0.1 or 0.001 g.
$Co(NO_3)_3$ 0.1, 0.02 or 0.2 g.
$ZnCl_2$ 0.2, 0.1, 0.05, 0.025 or 0.01 g.
$ZnSO_4$ 0.1, 0.5 or 1.0 g.
perosmic acid OsO_4 0.05, 0.01, 0.005, 0.5 or 0.1 g.

Author omitted the trace of $CaCl_2$.

Reference: Bokorny (1921 pp. 153–182).

386. Higgins' Basal Carbohydrate Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin	4.0 g.
3. Lactose	2.5 g.
4. Glucose	2.5 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add one of the combinations given under added nutrients.

(3) Adjust to neutral to phenolphthalein.

Sterilization: Method not given.

Use: Cultivation of organisms causing cholera and Picaton cattle disease.

Added nutrients: The following combinations were added:

(a)	Potassium phosphate	2.0 g.
	KNO_3	4.0 g.
(b)	Ammonium succinate	4.0 g.
	potassium phosphate	2.0 g.
	K_2SO_4	1.0 g.
	NH_4Cl	1.5 g.
(c)	Ammonium phosphate	2.5 g.
	KNO_3	2.5 g.
	KCl	1.5 g.
	$(NH_4)_2SO_4$	1.0 g.

Reference: Higgins (1898 pp. 666–667).

387. Peklo's Basal Asparagin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4	10.0 g.
3. $CaCl_2$	1.0 g.
4. $MgSO_4$	3.0 g.
5. $NaCl$	1.0 g.
6. Fe_2Cl_6 (Merck siccum)	trace
7. Mannitol	20.0 g.
8. Asparagin	4.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Add one of the added nutrients.

Sterilization: Generally sterilize all solutions for 30 minutes (method not given).

Use: Cultivation of plant actinomyces.

Added nutrients with modifications: The following combinations were added:

(a) The author used 1.0 g. KH_2PO_4, 0.1 g. $CaCl_2$, 0.3 g. $MgSO_4$ and 0.1 g. $NaCl$ and added 20.0 g. mannitol and 4.0 g. asparagin. The reaction was acid before sterilization. May be neutralized by the addition of Na_2CO_3.

(b) The author used 7.5 g. KH_2PO_4, and added 20.0 g. of mannitol and 4.0 g. asparagin. The reaction was slightly acid before sterilization. May be neutralized by the addition of Na_2CO_3.

(c) Add 100.0 cc. of the basal solution to 900.0 cc. of distilled water, and then add 20.0 g. mannitol and 4.0 g. asparagin. The reaction was weakly acid. The author specified that the solution might be neutralized by the addition of Na_2CO_3.

(d) Mix 250.0 cc. of the basal solution and add 750.0 cc. of distilled water, and add 20.0 g. mannitol and 4.0 g. asparagin. The action was distinctly acid.

(e) Mix 100.0 cc. of the basal solution with 900.0 cc. of distilled water. Add 30.0 g. mannitol and 4.0 g. asparagin. The reaction was weakly acid.

(f) Mix 250.0 cc. of the basal solution with 750.0 cc. of distilled water. Add 30.0 g. mannitol and 4.0 g. asparagin. The reaction was distinctly acid.

(g) The author used 5.0 g. KH_2PO_4 and 0.5 g. $CaCl_2$ and added 5.0 g. K_2HPO_4 to the basal solution. To this solution 20.0 g. mannitol and 4.0 g. asparagin were added. Reaction was distinctly acid. Filter the precipitate after the first sterilization. Sterilize for 90 minutes all together.

(h) The author used 5.0 g. KH_2PO_4 and 0.5 g. $CaCl_2$ and added 5.0 g. K_2HPO_4 to the basal solution. 100.0 cc. of this solution was diluted with 900.0 cc. of water, and 20.0 g. mannitol and 4.0 g. asparagin were added. Reaction was weakly acid. Filter the precipitate after the first sterilization. Sterilize for 90 minutes all together.

(i) The author used 5.0 g. KH_2PO_4, and 0.5 g. $CaCl_2$, omitted the $MgSO_4$ and NaCl from the basal solution and added 5.0 g. K_2HPO_4 and 3.0 g. $MgSO_4$. To 200.0 cc. of this solution added 800.0 cc. distilled water, 20.0 g. mannitol and 4.0 g. asparagin. The reaction is weakly acid. Sterilize for one hour.

Reference: Peklo (1910 p. 470).

388. Sullivan's Basal Glycerol Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol	10.0 g.
3. Asparagin	2.0 g.
4. K_2HPO_4	1.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add the various materials as indicated under added nutrients.

Sterilization: Not specified.

Use: To study pigment formation. The author reported that the blue-green pyocyanin was formed whenever there was present suitable combination of carbon hydrogen, oxygen and nitrogen and some salts to aid synthesis. For the formation of the fluorescent pigment, the presence of both sulphur and phosphorus was essential.

Added nutrients and variants:

(a) The author added 0.2 g. of $MgSO_4$ to the basal solution. Most chromogenic bacteria produced a pigment on this medium.

(b) Substituted 5.0 g. of asparagin for 2.0 g.

(c) Added 1.0 g. KNO_3.

(d) Used 20.0 g. glycerol, 3.0 g. asparagin and added 1.0 g. NaCl.

(e) Omitted the K_2HPO_4, used 10 to 20.0 g. glycerol, 10.0 g. asparagin, and added 0.2 to 1.0 g. KNO_3 and 0.2 to 1.0 g. NaCl.

(f) Used 10 to 20.0 g. glycerol, 10.0 g. asparagin and added 0.2 to 1.0 g. HBr.

(g) Used 20.0 g. asparagin, 20.0 g. glycerol and added 0.2 to 1.0 g. KI.

(h) Used 10.0 g. asparagin, 20.0 g. glycerol and made no additions, or added 0.2 to 1.0 g. KCl, 0.2 to 1.0 g. K_2SO_4, or 0.2 to 1.0 g. Na_2SO_4.

Reference: Sullivan (1905–06 p. 127–140).

389. Waksman's Basal Glycerol Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol	30.0 g.
3. K_2HPO_4	1.0 g.
4. KCl	0.5 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.01 g.
7. Asparagin	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Add 2.0 g. of one of the added nutrients.

(3) Tube in 10–12 cc. lots.

(4) Adjustment of reaction not specified.

Sterilization: Sterilize at 15 pounds for 15 minutes.

Use: To study metabolism of *Actinomycetes.*

Added nutrients: The author added 2.0 g. of one of the following:

$NaNO_3$ $(NH_4)_2SO_4$
$NaNO_2$ $(NH_4)_2CO_3$

Reference: Waksman (1920 p. 3).

390. Tanner's Basal Lactate Asparagin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. NaCl	5.0 g.
3. $CaH_4(PO_4)_2 \cdot H_2O$	2.0 g.
4. Ammonium lactate	6.0 g.
5. NaOH (normal)	20.0 cc.
6. Asparagin	4.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Add one of the listed added nutrients.
(3) Tube.

Sterilization: Sterilize in the Arnold Sterilizer.

Use: To study metabolism of sulphur. Production of H_2S by the fluorescent group, colon-typhoid group and others. H_2S production may be detected by the blackening of lead acetate paper.

Added nutrients: One of the following materials was employed:
Cystine (prepared from wool, Folin's method) 60.0 g.
2-thiohydantoin 1.0 cc. to each 5.0 cc. of medium.
Thio urea........................ 100.0 g.
Taurine, amount not given.
$Na_2S_2O_3 \cdot 5H_2O$.................. 3.0 g.
Na_2SO_3........................ 30.0 g.

Reference: Tanner (1917 p. 586).

391. Hiss' Basal Litmus Asparagin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	2.0 g.
3. $MgSO_4$	0.4 g.
4. NaCl	5.0 g.
5. Asparagin	4.0 g.
6. Ammonium lactate	5.0 cc.
7. Litmus (5.0% soln.)	15.0 cc.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Add 6.0 cc. of normal NaOH.
(3) Add 15.0 cc. of 5.0% litmus solution.
(4) Add 1.0% of one of the carbon sources given in added nutrients.
(5) The medium is clear.

Sterilization: Method not given.

Use: To study fermentation by the dysentery group. Acid production turns the medium from purplish blue to red.

Added nutrients: The author added 1.0% of one of the following:
glucose
maltose
sucrose
dextrin
mannitol

Reference: Hiss (1904 p. 32).

392. Harvey's Basal Lactate Asparagin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium lactate	5.0 g.
3. Na_2SO_4	2.0 g.
4. $MgSO_4$	0.2 g.
5. Asparagin	5.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: To ascertain production of indol test.

Added nutrients: Author added 30.0 g. of peptone, or 0.1, 0.3, or 0.5 g. of 1–1000 tryptophane.

Variants: The basic solution was used as culture medium without further additions.

Reference: Harvey (1921–22 p. 102).

393. Capaldi and Proskauer's Basal Citrate Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin	2.0 g.
3. $MgSO_4$	2.0 g.
4. Citric acid	5.0 g.
5. KH_2PO_4	2.0 g.
6. $CaCl_2$	0.2 g.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Add NaOH until the reaction is slightly alkaline.
(3) Add 0.2% of one of the carbon sources listed under added nutrients.

Sterilization: Not specified.

Use: To study fermentation by members of the colon-typhoid group. The authors reported no growth of *Bacterium typhi*. *B. coli* produced gas from glucose, levulose, lactose and mannose.

Added nutrients: 0.2% of one of the following was added:
glucose
levulose
lactose
sucrose
maltose
raffinose
maltose
mannose

Reference: Capaldi and Proskauer (1896 p. 454).

394. Mendel's Basal Citrate Asparagin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. KNO_3	2.0 g.
3. Asparagin	5.0 g.
4. $MgSO_4$	2.0 g.
5. Citric acid	5.0 g.
6. K_2HPO_4	2.0 g.
7. $CaCl_2$	0.2 g.
8. Iron chloride	

Preparation:

(1) Dissolve 2 and 3 in part of 1.

(2) Dissolve 4, 5, 6, 7 and 8 in the rest of 1.

(3) Neutralize (2) with KOH during the heating.

(4) Mix (3) and (1).

Sterilization: Sterilize for 20 minutes on each of two successive days. On the third day add 1.0% of one of the added nutrients and sterilize for 15 minutes.

Use: To study decomposition of sugars by *Bacterium coli* and *Bacterium Fitzianus.* The author reported the following reactions:

Variants: The author used 10.0 g. asparagin instead of 5.0 g. in the basic solution.

Added nutrients: The author employed 1.0% of one of the following as carbon sources:

glucose
maltose
lactose
sucrose

Reference: Mendel (1911 p. 297).

395. Maassen's Basal Malic Acid Asparagin Solution (Smith)

Constituents:

1.	Distilled water	1000.0	cc.
2.	Malic acid	7.0	g.
3.	Asparagin	10.0	g.
4.	Sodium phosphate (secondary)	5.0	g.
5.	$MgSO_4$	2.5	g.
6.	NaOH	2.5	g.
7.	$CaCl_2$	0.01	g.

Preparation:

(1) Dissolve 2 in 1000.0 cc. of distilled water.

(2) Neutralize exactly to litmus, using 7.0% KOH.

(3) Make up to 1000.0 cc.

(4) Dissolve 4, 5 and 6 in (3).

(5) Add 0.01 g. $CaCl_2$ to (4).

(6) Dissolve one of the added nutrients in (5).

Sterilization: Not specified.

Use: As a general culture medium.

Variants: Kolle and Wasserman used 0.4 g. $MgSO_4$ in the basal solution and 2.5 g. of pure crystalline soda instead of NaOH. He specified the use of 0.5 to 1.0% of any desired carbohydrate, alcohol, etc. as added nutrients.

Added nutrients: Smith added any desired carbohydrate, alcohol, etc.

Reference: Smith (1905 p. 198), Kolle and Wasserman (1912 p. 394).

396. Lockemann's Basal Citrate Glycerol Solution

Constituents:

1.	Water	1000.0	cc.
2.	$MgHPO_4 \cdot 7H_2O$	4.93	g.
3.	H_3PO_4 (1/3 molar)	20.0	cc.
4.	Citric acid (3 normal)	10.0	cc.
5.	Asparagin	5.29	g.
6.	Glycerol	20.0	g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Add one of the combinations given under added nutrients. The reaction of the various solutions will also be given there.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

Added nutrients and modifications: The author added the following materials:

(a) K_2HPO_4 0.35 g. and 10.0 cc. normal H_2SO_4. The acid titer was +4.7. Luxuriant growth was reported. (No indicator specified.)

(b) Used 5.0 g. asparagin, 12.0 cc. citric acid, 25.0 cc. H_3PO_4, 4.0 g. $MgHPO_4 \cdot 7H_2O$ and added 0.25 g. of $NaH_2PO_4 \cdot 12\,H_2O$, and 0.25 g. of KH_2PO_4. The acid titer was +4.0. Growth was reported as being quite good.

(c) Used 5.0 g. asparagin 12.0 cc. citric acid, 25.0 cc. of H_3PO_4, 3.75 g. $MgHPO_4 \cdot 7H_2O$ and added 0.25 g. $CaHPO_4$, 0.25 g. KH_2PO_4 and 0.25 g. NaH_2PO_4. The reaction was +4.2. Growth was reported as slight.

(d) Added 0.72 g. NaH_2PO_4, and 10.0 cc. normal H_2SO_4 to the basic solution. The acid titer was +4.7. Growth was reported as very slight.

(e) Added 10.0 cc. of normal H_2SO_4 and 0.35 g. K_2HPO_4 to the basic solution. The acid titer was +4.6. Growth reported as luxuriant.

(f) Added 10.0 cc. normal H_2SO_4, 0.18 g. K_2HPO_4 and 0.36 g. KH_2PO_4 to the basic solution. The acid titer was +4.2. Growth reported as being luxuriant.

Reference: Lockemann (1919 p. 421).

397. Mendel's Basal Citrate Glycerol Solution

Constituents:

1.	Distilled water	1000.0	cc.
2.	Asparagin	5.0	g.

3. Citric acid	0.75 g.
4. Glycerin	10.0 g.
5. Potassium biphosphate	5.0 g.
6. $MgSO_4$	2.5 g.
7. $(NH_4)_2SO_4$	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Adjustment of reaction not specified.

Sterilization: Sterilize for 30 minutes on each of two successive days. On the third day add 1.0% of one of the added nutrients and sterilize for 15 minutes.

Use: To study decomposition of sugars by *Bacterium coli* and *Bacterium Fitzianus.*

Added nutrients: The author added 1.0% of one of the following:

glucose	lactose
maltose	sucrose

Reference: Mendel (1911 p. 297).

398. Beijerinck and Minkman's Nitrate Glycocoll Solution

Constituents:

1. Water	1000.0 cc.
2. Glycocoll (2.5%)	25.0 g.
3. $NaNO_3$ (1.0%)	10.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: To study denitrification by soil forms, (*Bacillus nitroxus*). The medium was inoculated with soil.

Reference: Beijerinck and Minkman (1910 p. 37).

399. Bokorny's Sucrose Glycocoll Solution

Constituents:

1. Water	1000.0 cc.
2. Glycocoll	2.5 g.
3. Sucrose	50.0 g.
4. KH_2PO_4	2.0 g.
5. $MgSO_4$	1.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of Yeast.

Reference: Bokorny (1921 p. 336).

400. Hadley's Glycerol Glycocoll Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium lactate	7.5 g.
3. $FePO_4$	0.8 g.
4. Glycerin	30.0 g.
5. Glycocoll	1.0 g.
6. $MgSO_4$	3.2 g.
7. K_2HPO_4	2.3 g.
8. NaCl	6.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in sufficient water to make 1000.0 cc.

Sterilization: Not specified.

Use: Cultivation of *Bacillus diphtheriae* and study of toxin production.

Variants: The author omitted the $FePO_4$ and added 0.8 g. $CaCl_2$.

Reference: Hadley (1907 p. 101).

401. Beijerinck and Minkman's Nitrate Alanine Solution

Constituents:

1. Water	1000.0 cc.
2. Alanine (0.5%)	5.0 g.
3. KNO_3 (1.0%)	10.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Inoculate with soil.

Sterilization: Not specified.

Use: To study denitrification by soil forms, *Bacillus nitroxus.*

Reference: Beijerinck and Minkman (1910 p. 37).

402. Berthelot's Tyrosine Salt Solution

Constituents:

1. Water	1000.0 cc.
2. K_2SO_4	0.2 g.
3. $MgSO_4$	0.2 g.
4. K_2HPO_4	0.5 g.
5. KNO_3	0.25 g.
6. $CaCl_2$	0.02 g.
7. Tyrosine	2.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Enrichment and isolation—*Bacillus phenologenes. Bacillus phenologenes* is enriched in this medium and then plated on the same medium solidified with agar.

Reference: Berthelot (1913 p. 19).

403. Vansteenberge's Glucose Tyrosine Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose	20.0 g.
3. Tyrosine	0.2 g.
4. K_2HPO_4	0.5 g.
5. $MgSO_4$	2.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.
Use: Cultivation of *B. coli*.
Reference: Vansteenberge (1917 p. 609).

404. Bokorny's Sucrose Tyrosine Solution

Constituents:

1. Water	1000.0 cc.
2. Tyrosine	2.5 g.
3. Sucrose	50.0 g.
4. KH_2PO_4	2.0 g.
5. $MgSO_4$	1.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.
Sterilization: Not given.
Use: Cultivation of yeast.
Reference: Bokorny (1917 p. 340).

405. Waksman's Glycerol Tyrosine Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $MgSO_4$	0.5 g.
4. KCl	0.5 g.
5. $FeSO_4$	0.01 g.
6. Tyrosine	1.0 g.
7. Glycerol	30.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Tube in 10–12 cc. lots.
(3) Adjustment of reaction not specified.

Sterilization: Sterilize at 15 pounds for 15 minutes.
Use: To show utilization of tyrosine by *Actinomycetes*.
Reference: Waksman (1920 p. 18).

406. Myers' Cystine Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Cystine	5.0 g.

Preparation: (1) Dissolve the cystine in 1, using enough Na_2CO_3 to keep the cystine in solution.
Sterilization: Sterilize by filtration, then tube and test sterility.
Use: To study H_2S production. If H_2S is formed, lead acetate paper is blackened. No growth appeared with organisms studied unless medium was acid. Some organisms produce H_2S in acid medium.
Variant: The author gives the following variant:
(a) Add a bit of sterile litmus paper to each tube and sterile 5.0% HCl until the litmus is faintly red. The cystin is precipitated at neutrality.
(b) Inoculate and incubate.
(c) After a few days litmus is turned blue again and more HCl is added.
(d) Suspend a sterile piece of lead acetate soaked filter paper in each tube to test or H_2S production.

Reference: Myers (1918 p. 250).

407. Heap and Cadness' Cystine Solution

Constituents:

1. Distilled water	1000.0 cc.
2. NaCl	5.0 g.
3. Na_2HPO_4	4.2 g.
4. Sodium citrate	6.0 g.
5. Cystine	0.2 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.
Sterilization: Not specified.
Use: To study hydrogen sulphide production by *B. aertrycke*. The authors reported that the addition of glucose to this medium gave earlier and accelerated hydrogen sulphide production, but production ceases after 24 hours.
Reference: Heap and Cadness (1924–25 p. 86).

408. Rogers, Clark and Evans' Tryptophane Solution

Constituents:

1. Water	1000.0 cc.
2. Tryptophane	0.3 g.
3. K_2HPO_4	5.0 g.

Preparation:
(1) Dissolve 2 and 3 in 1.
(2) Tube in 10.0 cc. lots.

Sterilization: Not specified.
Use: To study production of indol. If indol is present a violet color appears when 1.0 cc. of a 2.0% alcoholic solution of p-dimethylamidobenzaldehyde and concentrated HCl are added (Zipfel's method).
Variants: Braun and Cahn-Bronner used 0.5% NaCl, 0.4% tryptophane, 0.2% K_2HPO_4 and added 0.7% normal $NaHCO_3$ after the solution was neutralized to litmus.
References: Rogers, Clark and Evans (1914 p. 101), Braun and Cahn-Bronner (1921 p. 199), Harvey (1921–22 p. 117).

409. Frieber's Glucose Tryptophane Solution

Constituents:

1. Water	1000.0 cc.
2. Tryptophane (0.03%)	0.3 g.

3. NaCl (0.5%)	5.0 g.
4. Potassium phosphate (0.2%)	2.0 g.
5. $MgSO_4$ (0.02%)	0.2 g.
6. Glucose (3.0%)	30.0 g.
7. $CaCO_3$	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Adjustment of reaction not given.

(3) Distribute in flasks in 50.0 cc. lots.

(4) Add 10.0% sterile $CaCO_3$ to each flask.

Sterilization: Not specified.

Use: To study indol production. The author reported that indol was not formed in the presence of glucose.

Reference: Frieber (1921–22 p. 268).

410. Bokorny's Sucrose Leucine Solution

Constituents:

1. Water	1000.0 cc.
2. Leucine	2.5 g.
3. Sucrose	50.0 g.
4. KH_2PO_4	2.0 g.
5. $MgSO_4$	1.0 g.

Preparation: (1) Dissolve one of 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: To study the nutrient requirements for growth of yeast.

Reference: Bokorny (1917 p. 340).

411. Dolt's Ammonium Asparagin Solution No. I

Constituents:

1. Distilled water	1000.0 cc.
2. Asparagin	10.0 g.
3. $(NH_4)_2HPO_4$	2.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Make neutral to phenolphthalein with NaOH.

Sterilization: Not specified.

Use: Cultivation of colon group and organisms found in water.

Reference: Dolt (1908 p. 620), Tanner (1919 p. 65).

412. Kendall, Walker and Day's Ammonium Asparagin Solution

Constituents:

1. Redistilled water	1000.0 cc.
2. Asparagin	2.0 g.
3. $(NH_4)_2HPO_4$	2.0 g.
4. Na_2HPO_4	1.0 g.
5. NaCl	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Adjustment of reaction not given.

(3) Distribute in 100.0 cc. lots.

Sterilization: Method not given.

Use: To study lipolytic and proteolytic activity by the tubercle bacilli. To determine lipolytic action, suspend 1.0 cc. of the bacteria free filtrate of the culture in freshly boiled distilled water. Add 0.25 cc. of ethylbutyrate and 0.5 cc. of toluene and incubate at 37°C. The increase in acidity as measured in terms of N/50 NaOH measures lipolytic activity.

Variants: The authors used 4.0 g. asparagin and omitted the Na_2HPO_4.

Reference: Kendall, Walker and Day (1914 p. 463).

413. Dolt's Asparagin Solution No. 2

Constituents:

1. Distilled water	1000.0 cc.
2. Asparagin	10.0 g.
3. Na_2HPO_4	2.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Neutralize with NaOH using phenolphthalein as an indicator.

Sterilization: Method not given.

Use: Cultivation of colon group and organisms found in water.

Variants: Dolt added 5.0 g. NaCl and neutralized the medium by the addition of $Na_2CO._3$

References: Dolt (1908 pp. 617, 620), Tanner (1919 p. 65).

414. Beijerinck and Minkman's Nitrate Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin (0.05%)	0.5 g.
3. KNO_3 (1.0%)	10.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Inoculate with soil.

Sterilization: Not specified.

Use: To study denitrification by soil forms, *Bacillus pyocyaneus*.

Variants: Author employed the above medium with the addition of 5.0 g. K_2HPO_4.

Reference: Beijerinck and Minkman (1910 p. 37).

415. Blanchetiére's Asparagin Salt Solution

Constituents:

1. Distilled water	900.0 cc.
2. NaCl	5.0 g.
3. Na_2HPO_4	1.0 g.
4. Potassium biphosphate	1.0 g.
5. Asparagin	3.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in enough water to make 900.0 cc.

(2) Distribute in 450.0 cc. lots in liter flasks.

Sterilization: Sterilize for 15 minutes at 110° to 112°C.

Use: To study asparagin decomposition by *Bacillus fluorescens liquefaciens* (Flügge).

Reference: Blanchetiére (1917 p. 294).

416. Park, Williams and Krumwiede's Asparagin Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin	6.0 g.
3. Sodium phosphate (ortho)	2.0 g.
4. NaCl	5.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) If necessary make alkaline to litmus by the addition of NaOH.

(3) Tube in small quantities.

(4) Test for color production with. *B. pyocyaneus*.

Sterilization: Method not given.

Use: Culture medium for disinfection tests.

Reference: Park, Williams and Krumwiede (1924 p. 122).

417. Bokorny's Asparagin Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin (0.5%)	5.0 g.
3. KH_2PO_4 (0.1%)	1.0 g.
4. $MgSO_4$ (0.03%)	0.3 g.
5. $CaCl_2$	trace
6. K_2SO_4 (0.1%)	1.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study effect of metallic salts on yeast growth. The author reported that 4.0% of K_2SO_4 did not inhibit the development of the yeasts.

Variants: The author used 0.5, 1.0, 2.0 or 4.0% K_2SO_4.

Reference: Bokorny (1912 p. 122).

418. Uschinsky's Asparagin Salt Solution (Giltner)

Constituents:

1. Distilled water	1000.0 cc.
2. Asparagin	3.4 g.
3. NaCl	5.0 g.
4. $MgSO_4$	0.2 g.
5. $CaCl_2$	0.1 g.
6. KH_2PO_4	1.0 g.
7. $FeSO_4$	trace

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: General culture medium.

Variants:

(a) Gottheil did not specify the type of phosphate used and used 0.3 g. $MgSO_4$ with 10.0 g. asparagin.

(b) Linde used 0.01 g. Fe_2Cl_6 instead of $FeSO_4$, used 0.3 g. $MgSO_4 \cdot 7H_2O$, 0.1 g. NaCl, and did not specify the amount of asparagin added. H used the solution with an alkaline reaction for the cultivation of Cladothrix. The addition of glucose, sucrose or glycerol would have increased the amount of growth.

References: Giltner (1921 p. 369), Gottheil (1901 p. 432), Linde (1913 p. 386).

419. Henneberg's Asparagin Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Asparagin (0.075 to 0.9%)	0.75 to 9.0 g.
3. K_2HPO_4 (0.05 to 1.5%)	0.5 to 15.0 g.
4. $MgSO_4$ (0.02 to 0.6%)	0.2 to 6.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of yeast.

Variants:

(a) Bokorny used 25.0 g. asparagin, 5.0 g. KH_2PO_4 and 1.0 g. $MgSO_4$.

(b) Evans used 5.0 g. asparagin, 1.0 g. $MgSO_4$ and 2.0 g. K_2HPO_4.

(c) Heinemann and Tanner used 2.0 g. asparagin, 1.0 g. $MgSO_4$ and 1.0 g. K_2HPO_4.

(d) Tanner used 10.0 g. asparagin, 2.0 g. $MgSO_4$ and 1.0 g. KH_2PO_4 for pigment production by *Ps. pyocyaneus* and *Ps. fluorescens liquefaciens*.

(e) Boehncke used 2.0% asparagin, 0.5%

NaCl and 0.05% $MgSO_4$ to determine ammonia production by Proteus.

References: Henneberg (1907 pp. 40–45), (1909 p. 104), Bokorny (1917 p. 338), Evans (1916 p. 444), Heinemann (1922 p. 42), Tanner (1919 pp. 64, 66), Boehncke (1911 p. 74).

420. Arnaud and Charrin's Asparagin Salt Solution (Roux and Rochaix)

Constituents:

1. Water	1000.0	cc.
2. KH_2PO_4	0.1	g.
3. $Na_2HPO_4 \cdot 12H_2O$	0.1	g.
4. $KHCO_3$	0.134	g.
5. $CaCl_2$	0.05	g.
6. $MgSO_4 \cdot 7H_2O$	0.05	g.
7. Asparagin	5.0	g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Cultivation of *Bacillus pyocyaneus*.

Reference: Roux and Rochaix (1911 p. 104).

421. Saltet's Glucose Asparagin Solution

Constituents:

1. Distilled water	1000.0	cc.
2. NaCl	12.0	g.
3. KCl	1.0	g.
4. Na_2HPO_4	0.40	mg.
5. Glucose	0.5	g.
6. Asparagin	1.0	g.
7. Na_2SO_4	varying amounts	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Add varying amounts of Na_2SO_4 (amount not given) to (1).

Sterilization: Not specified.

Use: To study sulphate reduction by *B. desulfuricans*. Sulphates were reduced.

Variants: The author substituted $MgSO_4$ for Na_2SO_4.

Reference: Saltet (1900 p. 699).

422. Saltet's Thiosulphate Asparagin Solution

Constituents:

1. Distilled water	1000.0	cc.
2. NaCl	12.0	g.
3. KCl	1.0	g.
4. Na_2HPO_4	30.0	mg.
5. Na_2SO_4	1.0	g.
6. $Na_2S_2O_3$	1.5	g.
7. Asparagin	1.0	g.
8. Glucose	1.5	g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: To study thiosulphate reduction by *B. desulfuricans* and *B. coli*. When ammonia and sodium nitroprusside were added to either culture a negative reaction was obtained.

Reference: Saltet (1900 p. 702).

423. Blanchetiére's Glucose Asparagin Solution

Constituents:

1. Distilled water	1000.0	cc.
2. NaCl	5.0	g.
3. Na_2HPO_4	1.0	g.
4. K_2HPO_4	1.0	g.
5. Asparagin	5.0	g.
6. Glucose	3.0	g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in enough water to make 1000.0 cc.

(2) The reaction is alkaline to litmus before sterilization and neutral after sterilization.

Sterilization: Autoclave at 112° for 40 minutes.

Use: To study asparagin decomposition by *Bacillus fluorescens liquefaciens* (Flügge). Good growth when NH_4Cl is added but no pigment is produced.

Variants: The author specified that 5.0 g. NH_4Cl might be added.

Reference: Blanchetiére (1917 p. 301).

424. Capaldi and Proskauer's Glucose Asparagin Solution

Constituents:

1. Water	1000.0	cc.
2. Asparagin (0.2%)	2.0	g.
3. NaCl (0.02%)	0.2	g.
4. $MgSO_4$ (0.01%)	0.1	g.
5. $CaCl_2$ (0.02%)	0.2	g.
6. KH_2PO_4 (0.2%)	2.0	g.
7. Dextrose (0.2%)	2.0	g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Acid production by *Bacterium coli* and typhoid bacillus.

Variants: Robertson and Davis used the following solution to study the influence of vitamines on bacterial growth. All materials were chemically pure. This medium did not give continued growth of yeast.

1. Sterile distilled water	1000.0 cc.
2. Asparagin (Merck)	3.4 g.
3. $CaCl_2$	0.1 g.
4. Dextrose	20.0 g.
5. $MgSO_4$	0.2 g.
6. K_2HPO_4	1.0 g.
7. NaCl	5.0 g.

References: Capaldi and Proskauer (1896 p.456), Robertson and Davis (1923 p. 154).

425. Gottheil's Glucose Asparagin Solution No. X

Constituents:

1. Distilled water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $CaCl_2$	1.0 g.
4. $MgSO_4$	0.3 g.
5. NaCl	0.1 g.
6. Iron	trace
7. Asparagin	10.0 g.
8. Glucose	30.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: Cultivation of organisms found in the soil, on roots and on rhizomes.

Reference: Gottheil (1910 p. 432).

426. Capaldi and Proskauer's Glucose Asparagin Solution No. II

Constituents:

1. Water	1000.0 g.
2. Asparagin	2.0 g.
3. Glucose	2.0 g.
4. NaCl	5.0 g.
5. $MgSO_4$	2.0 g.
6. Sodium phosphate	2.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Add NaOH until the reaction is slightly alkaline.

Sterilization: Not specified.

Use: Acid production by *Bacterium coli* and typhoid bacilli.

Reference: Capaldi and Proskauer (1896 p. 456).

427. Stutzer's Glucose Asparagin Solution

Constituents:

1. Water	1000.0 g.
2. Glucose	10.0 g.
3. Asparagin	2.0 g
4. KH_2PO_4	0.5 g.
5. $MgSO_4$	0.25 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of bacteroids from *Pisum sativum.*

Variants:

(a) Hefferan used 1.0 g. $MgSO_4$, 1.0 g. sodium phosphate and 20.0 g. of glucose to cultivate *B. rosaceus metalloides.* This organism forms rosette-like groupings in liquid media. The addition of glucose aids the growth of the organism.

(b) Kuntze used the following solution to study pigment production by *Bacillus ruber indicus.* He reported that the organism produced pigment when grown in medium without $MgSO_4$ for 6 days and then in medium containing $MgSO_4$.

1. Distilled water	1000.0 cc.
2. d-glucose (Soxhlet)	25.0 g.
3. Asparagin	10.0 g.
4. K_2HPO_4	2.0 g.
5. $MgSO_4$	0.0 or 2.0 g.

(c) Kuntze used the following solution to study pigment production by *Aspergillus niger* and *Bacillus prodigiosus.*

1. Distilled water	1000.0 cc.
2. $MgSO_4$	1.0 to 2.0 g.
3. K_2HPO_4	2.0 g.
4. d-glucose	25.0 g.
5. Asparagin	10.0 g.

(d) Kuntze used the following solution to study pigment production by *Bacillus prodigiosus.* He reported that when KH_2PO_4 was used pigment was produced:

1. Distilled water	1000.0 cc.
2. Asparagin	10.0 g.
3. d-glucose	20.0 g.
4. KH_2PO_4 or K_2HPO_4	2.0 g.
5. $MgSO_4$	2.0 g.

(e) Zikes used a solution containing 100.0 g. glucose, 2.5 g. asparagin and 3.3 g. $MgSO_4$ per liter to determine volutin production by *Oidium lactis.* He distributed the solution in 10.0 cc. quantities and added 0.25, 0.125, 0.063, 0.032 or 0.016 g. K_2HPO_4 to each tube. He reported that decreasing the K_2HPO_4 content decreased volutin production.

References: Stutzer (1900 p. 901), Hefferan (1902 p. 694), Kuntze (1907 pp. 301, 304, 307), Zikes (1922 p. 33).

428. Saltet's Magnesium Sulphate Asparagin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. NaCl	12.0 g.
3. KCl	1.0 g.
4. Na_2HPO_4	0.03 g.
5. $MgSO_4$	1.0 g.
6. Na_2SO_3	1.5 g.
7. Glucose	0.5 g.
8. Asparagin	1.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: To study sulphite reduction by *B. desulfuricans.* No H_2S reaction when using sodium nitroprusside to detect H_2S.

Reference: Saltet (1900 p. 697).

429. Samkow's Glucose Asparagin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Asparagin	10.0 g.
3. Glucose	5.0 g.
4. KH_2PO_4	2.0 g.
5. Na_2CO_3	2.5 g.
6. $MgSO_4$	0.4 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study pigment production by *Bacillus prodigiosus.* A red pigment was produced.

Reference: Samkow (1903 p. 306).

430. Sullivan's Glucose Asparagin Solution

Constituents:

1. Water	1000.0 g.
2. Asparagin	10.0 g.
3. Dextrose	5.0 g.
4. $MgSO_4$	0.3 g.
5. K_2HPO_4	1.0 g
6. KNO_3	1.0 g.
7. NaCl	1.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: As a general culture medium, and also used to study pigment production.

Reference: Sullivan (1905–06 p. 115).

431. Boehncke's Glucose Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin (5.0%)	50.0 g.
3. NaCl (0.5%)	5.0 g.
4. Potassium biphosphate (0.2%)	2.0 g.
5. $MgSO_4$ (0.05%)	0.5 g.
6. Dextrose (1.0 or 2.0%)	10.0 or 20.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To determine the formation of ammonia by *Proteus* types.

Reference: Boehncke (1911 p. 103).

432. Zikes' Sucrose Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	5.0 g.
3. K_2SO_4	2.5 g.
4. Asparagin	10.0 g.
5. Sucrose	75.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: To study perithecium formation by *Aspergillus oryzae.*

Reference: Zikes (1922 p. 340).

433. Korffs' Sucrose Asparagin Solution

Constituents:

1. Water	
2. KH_2PO_4	25.0 g.
3. $MgSO_4$ (crystalline)	8.5 g.
4. Asparagin	20.0 g.
5. Sugar candy	

Preparation:

(1) Dissolve 2, 3 and 4 in 1 liter of water.
(2) Dissolve 1000.0 g. of white sugar candy in 500.0 cc. distilled water by heating on the water bath.
(3) Filter while hot in a hot water funnel into a large porcelain dish.
(4) Add 2 liters of warm absolute alcohol, stirring constantly.
(5) Pour the cool alcohol from the sugar and wash the sugar with absolute alcohol and then with ether.

(6) Dry at 60°C. until all traces of dampness of alcohol or ether have disappeared.
(7) Prepare a 10.0% solution of (6) in distilled water.
(8) Mix about 10.0% of (7) with 10.0% of (1).
(9) Distribute in 150.0 cc. lots in fermentation flasks.

Sterilization: Sterilize in streaming steam.

Use: To study fermentation by yeast.

Reference: Korff (1898 p. 532).

434. Zikes' Magnesium Sucrose Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin	10.0 g.
3. K_2SO_4	5.0 g.
4. $MgSO_4$	2.5 g.
5. Saccharose	75.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: To study perithecium formation by *Aspergillus oryzae*.

Reference: Zikes (1922 p. 342).

435. Chrzaszcz's Sucrose Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Sucrose	100.0 g.
3. $Mg(H_2PO_4)_2$	2.0 g.
4. $Ca(H_2PO_4)_2$	2.0 g.
5. K_2SO_4	2.0 g.
6. Asparagin	5.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study growth requirements for yeasts. Author reported that yeast developed very well in this medium.

Reference: Chrzaszcz (1904 p. 149).

436. Sullivan's Glycerol Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin	10.0 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4$	0.2 g.
5. Glycerol	20.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of chromogenic bacteria. The author reported that the chromogenic bacteria developed rapidly with good color production on this medium.

Variants: (1) Bezançon used 2.5 g. $MgSO_4$, 1.5 g. potassium phosphate, 4.0 g. asparagin and 15.0 g. glycerol. This solution was used for the cultivation of tubercle bacilli.

Reference: Sullivan (1905–06 p. 116), Bezançon (1920 p. 547).

437. Schweinitz and Dorset's Glycerol Asparagin Solution (Goris)

Constituents:

1. Water	1000.0 cc.
2. Glycerol	70.0 g.
3. KH_2PO_4	1.0 g.
4. Ammonium phosphate	10.0 g.
5. NaCl	10.0 g.
6. Asparagin	2.0 g.
7. $MgSO_4$	2.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

Reference: Goris (1920 p. 499).

438. Waksman and Joffe's Glycerol Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol	30.0 g.
3. $MgSO_4$	0.5 g.
4. KCl	0.5 g.
5. $FeSO_4$	0.01 g.
6. Asparagin	5.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Adjust medium to different pH from 4.4 to 8.7 with phosphates and carbonates.
(3) Tube in 10–12 cc. lots.

Sterilization: Sterilize at 15 pounds for 15 minutes.

Use: To study effect of reaction (pH) on metabolism of actinomycetes.

Reference: Waksman and Joffe (1920 p. 44).

439. Hadley's Glycerol Asparagin Solution Nos. III and IV

Constituents:

1. Water	1000.0 cc.
2. Ammonium phosphate	10.0 g.

3. Asparagin	10.0 g.
4. $CaCl_2$	0.1 g.
5. Glycerin	40.0 g.
6. KNO_3	0.3 g.
7. K_2HPO_4	0.3 g.
8. NaCl	6.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: Cultivation of *Bacillus diphtheriae* and study of toxin production.

Variants: The author used 0.3 g. $MgSO_4$ instead of 0.6 g. NaCl for solution No. 4.

Reference: Hadley (1907 p. 101).

440. Peklo's Mannitol Asparagin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4	15.0 g.
3. $CaCl_2$	1.0 g.
4. $MgSO_4$	3.0 g.
5. NaCl	1.0 g.
6. Fe_2Cl_6 (Merck siccum)	
7. Mannite	20.0 g.
8. Asparagin	4.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 (amount of 6 not given) in 1.

(2) Neutralize with K_2CO_3.

(3) Partially correct the precipitate loss by the addition of orthophosphoric acid.

(4) Dissolve 7 and 8 in (3).

(5) Reaction is found to be neutral after sterilization.

Sterilization: Method not given.

Use: Cultivation of plant actinomyces.

Variants:

(a) Peklo prepared the following solution:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	5.0 g.
3. KH_2PO_4	4.5 g.
4. $MgSO_4$	3.0 g.
5. NaCl	1.0 g.
6. $CaCl_2$	1.0 g.
7. Fe_2Cl_6	trace

He diluted 200.0 cc. of this solution with 800.0 cc. of distilled water and added 20.0 g. mannitol and 4.0 g. asparagin. This solution was sterilized twice for one hour. The final reaction is weakly acid.

(b) Peklo added 20.0 g. of mannitol, 4.0 g. asparagin and 100.0 cc. of Meyers solution (preparation not given) to 900.0 cc. of distilled water. The mixture was boiled 30 minutes. Add soda until the reaction is strongly alkaline.

Reference: Peklo (1910 pp. 509, 510, 522).

441. Capaldi and Proskauer's Mannitol Asparagin Solution I

Constituents:

1. Distilled water	1000.0 cc.
2. Asparagin (0.2%)	2.0 g.
3. Mannite (0.2%)	2.0 g.
4. NaCl (0.02%)	0.2 g.
5. $MgSO_4$ (0.01%)	0.1 g.
6. $CaCl_2$ (0.02%)	0.2 g.
7. KH_2PO_4 (0.2%)	2.0 g.
8. Litmus.	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Steam for 30 minutes in the steamer.

(3) Add NaOH to obtain a neutral reaction to litmus.

(4) Add litmus to color.

(5) Sterilize again for 30 minutes in the steamer.

(6) Filter.

(7) Tube in 5.0 cc. lots.

(8) Plug the tubes with cotton.

Sterilization: Final sterilization not specified.

Use: Differentiation of colon-typhoid group of bacteria. *B. coli* produced acid, typhoid bacillus produced no change.

Variants: Harvey used 2.0 g. NaCl.

Reference: Capaldi and Proskauer (1896 p. 472), Harvey (1921–22 p. 102).

441a. Ellrodt's Acetate Asparagin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Asparagin	5.0 g.
3. Sodium acetate	5.0 g.
4. Potassium phosphate	2.0 g.
5. NaCl	2.0 g.
6. $MgSO_4$	0.1 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not given.

Use: Cultivation of *Bact. pyocyaneum.*

Reference: Ellrodt (1902 p. 641).

441b. Voges' Lactate Asparaginate Solution

Constituents:

1. Distilled water	200.0 cc.
2. NaCl	8.0 g.
3. K_2HPO_4	2.0 g.
4. Ammonium lactate	6.0 g.
5. Sodium asparaginate	4.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Method not given.

Use: Enrichment and isolation of cholera bacilli from water. Add 800.0 cc. of the water to be investigated to the medium. Used also as a general culture medium.

Variants:

(a) Sames used 5.0 g. NaCl, 4.0 g. asparagin and 8.0 g. ammonium lactate. He distributed the solution in 5.0 cc. lots and added 0.0, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1.1 cc. of a 5.0% NaOH solution to each tube. He reported that motile sarcine grew best when 0.25 to 0.3 cc. of 5.0% NaOH solution was added to each 5.0 cc. medium.

(b) Heinemann used 2.0 g. Na_2HPO_4 instead of K_2HPO_4, 5.0 g. NaCl, 6.3 g. ammonium lactate and 3.4 g. asparagin.

(c) Kuntze used 2.0 g. KH_2PO_4 instead of K_2HPO_4, 4.0 g. asparagin and 5.0 g. NaCl. He used this medium to study pigment production by *Bacillus prodigiosus*.

(d) Dolt used 5.0 g. NaCl, 2.0 g. Na_2HPO_4 instead of K_2HPO_4 and 4.0 g. asparagin. The solution was neutralized by the addition of Na_2CO_3.

(e) Nicolle, Raphael and Debaiens used 5.0 g. NaCl and 4.0 g. of asparagin.

(f) Magnusson used 5.0 g. NaCl. The medium was used to study slime production by *Bacterium lactic viscosum* and streptococci.

(g) Tanner used 5.0 g. NaCl.

(h) Bezançon used from 5.0 to 7.0 g. NaCl, 6.0 to 7.0 g. ammonium lactate and 3.0 to 4.0 g. asparagin.

(i) Abbott, Dopter and Sacquépée, and also Park, Williams and Krumuriede used 4.0 g. asparagin, 5.0 g. NaCl, and 2.0 g. Na_2HPO_4. Abbott cultivated *Pseudomonas aeruginosa*, the bacillus of green pus, on this medium.

References: Voges (1894 p. 453), Sames (1898 p. 665), Heinemann (1905 p. 131), Kuntze (1907 p. 300), Dolt (1908 p. 617), Nicolle, Raphael and Debaiens (1917 p. 378), Magnusson (1918 p. 467), Tanner (1919 p. 68), Bezançon (1920 p. 116), Abbott (1921 p. 385), Dopter and Sacquépée (1921 p. 122), Park, Williams and Krumwiede (1924 p. 122).

441c. Fraenkel and Voges' Salt Asparagin Solution (Besson)

Constituents:

1. Water	1000.0 cc.
2. NaCl	5.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.2 g.
5. K_2HPO_4	2.0 g.
6. Ammonium lactate	6.0 g.
7. Asparagin	3.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Neutralize.

Sterilization: Method not given.

Use: General culture medium.

Reference: Besson (1920 p. 37).

442. Frankel's Lactate Asparagin Solution (Tanner)

Constituents:

1. Distilled water	1000.0 cc.
2. NaCl	5.0 g.
3. $CaHPO_4$	2.0 g.
4. Ammonium lactate	6.0 g.
5. Asparagin	4.0 g.
6. NaOH (normal)	20.0 cc.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Filter.

Sterilization: Sterilize in Arnold on 3 successive days.

Use: For cultivation of green fluorescent bacteria. This medium aided in pigment production of strains studied.

Variants:

(a) Tanner used 2.0 g. $Ca(HPO_4)_2$ instead of $CaHPO_4$.

(b) Löhnis used 2.0 g. of K_2HPO_4, $Ca(HPO_4)_2$ and made distinctly alkaline by the addition of NaOH, instead of adding 20.0 cc. of normal NaOH.

References: Tanner (1918 p. 82), (1919 p. 64), Löhnis (1913 p. 44), Klimmer (1923 p. 173).

443. Uschinsky's Lactate Asparaginate Solution (Smith)

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium lactate	5.0 g.
3. Sodium asparaginate	2.5 g.
4. $Na_2SO_4 \cdot 10H_2O$	2.5 g.
5. NaCl	2.5 g.
6. K_2HPO_4	2.5 g.
7. $CaCl_2$	0.01 g.
8. $MgSO_4$	0.01 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: As a general culture medium.

References: Smith (1905 p. 197), Tanner (1919 p. 69).

444. Melick's Lactate Asparagin Solution

Constituents:

1. Redistilled water	1000.0 cc.
2. NaCl	5.0 g.
3. Asparagin	3.4 g.
4. Na_2HPO_4	2.0 g.
5. $MgSO_4$	0.5 g.
6. Ammonium lactate	6.3 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Adjust to +0.2 acid by the addition of Na_2CO_3.

Sterilization:

(1) Autoclave for 10 minutes at 10 pounds pressure.

(2) Tube and autoclave again at 10 pounds pressure for 10 minutes. Discard all tubes showing a precipitate.

Use: Growth of organisms for preparation of bacterial antigens, in colon-typhoid group.

Reference: Melick (1922 p. 408).

445. van Delden's Lactate Ammonia Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl	30.0 g.
3. K_2HPO_4	0.5 g.
4. $MgSO_4 \cdot 7H_2O$	2.5 g. (or more)
5. Sodium lactate	5.0 to 10.0 g.
6. Asparagin	1.0 g.
7. Mohr's salt ($FeSO_4(NH_4)_2SO_4$).	trace
8. Na_2SO_3	small quantity

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

(2) Inoculate with sea water or sand from a beach.

Sterilization: Not specified.

Use: Enrichment of sulphite and sulphate reducers from sea water, *Microspira aestuarii*.

Variants: Omit the Na_2SO_3 if inoculation is made with sea mud colored black with H_2S.

Reference: van Delden (1903–04 p. 93).

446. Beijerinck's Lactate Asparagin Solution

Constituents:

1. Water (ditch)	1000.0 cc.
2. Asparagin	2.5 g.
3. $MgSO_4$	2.5 g.
4. Potassium phosphate	2.0 g.
5. Iron lactate	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in ditch water ("Graben wasser").

(2) Make alkaline with Na_2CO_3.

Sterilization: Method not given.

Use: To study the reduction of sulphates. Author reported strong H_2S and ammonium sulfide production after 48 hours at 30°C.

Reference: Beijerinck (1895 p. 107).

447. van Delden's Lactate Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	0.5 g.
3. Sodium lactate	5.0 g.
4. Asparagin	1.0 g.
5. $MgSO_4 \cdot 7H_2O$	1.0 g.
6. $FeSO_4$	trace

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not given.

Use: Sulphate reduction by *Spirillum* (*Microspira*) *desulfuricans*. Medium turned dark due to H_2S production. H_2S may also be determined by titration with 0.01 N iodine solution.

Variants:

(a) The author substituted 1.0 g. gypsum for $MgSO_4$.

(b) Tanner and Giltner specified the use of tap water.

References: van Delden (1903–04 p. 83), Tanner (1919 p. 64), Giltner (1921 p. 377).

448. Schroeder and Junger's Lactate Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin	4.0 g.
3. Sodium lactate	6.0 g.
4. Sodium phosphate (Ortho)	2.0 g.
5. NaCl	5.0 g.

Preparation:

(1) Powder 2, 3, 4 and 5 separately.
(2) Dissolve the powdered salts separately in 50 to 100.0 cc. of water.
(3) Mix the solutions and add the remainder of the liter of water.
(4) Add NaOH to make slightly alkaline to litmus.
(5) Filter.
(6) Tube.

Sterilization: Sterilize on 2 consecutive days in the Arnold sterilizer.

Use: Cultivation of *Bacillus pyogenes* and chromogenes. The author reported that a green band of pigment was produced after 24 hours. The band gradually increased until the whole tube was light green. Non chromogenic organisms were inhibited.

Variants: Harvey used 2.0 g. of Na_2HPO_4.

References: Schroeder and Junger (1912 p. 601), Harvey (1921–22 p. 102).

449. Remy's Organic Acid Asparagin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Asparagin	6.0 g.
3. Oxalic acid	0.5 g.
4. Lactic acid	0.15 g.
5. Citric acid	0.15 g.
6. Na_2HPO_4	5.0 g.
7. $MgSO_4$	2.5 g.
8. K_2SO_4	1.25 g.
9. NaCl	2.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

Sterilization: Not specified.

Use: Isolation of typhoid bacilli.

Reference: Thoinot and Masselin (1902 p. 337).

450. Kuntze's Citrate Asparagin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. KNO_3	2.0 g.
3. Asparagin	1.0 g.
4. $MgSO_4$	2.0 g.
5. Citric acid	5.0 g.
6. KH_2PO_4	2.0 g.
7. $CaCl_2$	0.2 g.
8. Iron chloride	several drops

Preparation:

(1) Dissolve 2 and 3 in part of 1.
(2) Dissolve 4, 5, 6, 7 and 8 in the remainder of 1.
(3) Neutralize (2) with KOH during the heating.
(4) Mix (3) and (1).
(5) Distribute into 100.0 cc. lots in combustion flasks.

Sterilization: Method not given.

Use: To study morphology and physiology of denitrifying organisms *Bacillus denitrificans agilis* (*Ampola and Garino*) *and Bacillus oxalaticus* (Zopf).

Variants:

(a) Wojtkiewicz used 3.0 g. KNO_3, 7.6 potassium citrate instead of citric acid, used 0.2 g. $CaCl_2$ and specified a trace of $FeCl_2$.
(b) Arnd and Harvey specified a trace of $FeCl_2$.

References: Kuntze (1904 p. 3), Smith (1905 p. 198), Wojtkiewicz (1914 p. 258), Arnd (1916 p. 567), Harvey (1921–22 p. 102).

451. Giltay and Aberson's Citrate Asparagin Solution (Tanner)

Constituents:

1. Distilled water	1000.0 cc.
2. KNO_3	2.0 g.
3. $MgSO_4$	2.0 g.
4. Citric acid	5.0 g.
5. K_2HPO_4	2.0 g.
6. $CaCl_2$	0.2 g.
7. Na_2CO_3	4.25 g.
8. Asparagin	1.0 g.

Preparation:

(1) Dissolve 2 and 8 in a little distilled water.
(2) Dissolved 3, 4, 5, 6 and 7 in distilled water.

(3) Mix (1) and (2) and make up to a liter.
(4) Tube in fermentation tubes.
(5) Adjustment of reaction not specified.

Sterilization: Method not given.

Use: Cultivation of denitrifying bacteria.

Variants: Löhnis used 2.0 g. KH_2PO_4 instead of K_2HPO_4, omitted the Na_2CO_3 and added a trace of Fe_2Cl_6.

References: Tanner (1919 p. 67), Löhnis (1913 p. 97).

452. Maassen's Basal Citrate Asparagin Solution (Löhnis)

Constituents:

1. Distilled water	1000.0	cc.
2. Citric acid	7.0	g.
3. Asparagin	10.0	g.
4. K_2HPO_4	2.0	g.
5. Na_2CO_3 (crystalline)	2.5	g.
6. $MgSO_4$	0.4	g.
7. $CaCl_2$	0.01	g.

Preparation:
(1) Neutralize 7.0 g. citric acid with pure KOH.
(2) Dissolve (1), 3, 4, 5, 6 and 7 in 1.
(3) Add from 1.5 to 4.0% of one of the nutrients listed below to (2).

Sterilization: Sterilize on each of 3 successive days.

Use: As a general synthetic medium.

Added nutrients: Löhnis added from 1.5 to 4.0% of one of the following materials:
sugars
alcohols
other sources of carbon

Reference: Löhnis (1913 p. 44).

453. Maassen's Malate Asparagin Solution

Constituents:

1. Distilled water	1000.0	cc.
2. Malic acid	7.0	g.
3. KOH		
4. Asparagin	10.0	g.
5. $MgSO_4$	0.4	g.
6. Na_2HPO_4	2.0	g.
7. Soda	2.5	g.
8. $CaCl_2$	0.01	g.

Preparation:
(1) Dissolve 7.0 g. malic acid in about 100.0 cc. distilled water.
(2) Neutralize (1) with KOH.
(3) Make volume to 1000.0 cc.
(4) Dissolve 10.0 g. finely powdered asparagin, 0.4 g. $MgSO_4$, 2.0 g. Na_2HPO_4 and 2.5 g. crystalline pure soda.

Sterilization: Method not given.

Use: Cultivation of cholera vibrio.

Variants: The malic acid may be replaced by 1/10 equivalent amount of any utilizable organic acid. Sodium may replace potassium. The ammonia salt of any inorganic or organic acid may replace the asparagin or the asparagin may be replaced by different nitrogenous organic compounds such as amides, amino acids, urea, or creatinin. The amount of soda added may be changed and the amount of water added may be increased. The addition of cane sugar, lactose, maltose, galactose, dextrose or dextrin gives a more luxuriant growth.

References: Massen (1894 pp. 401, 404), also abstract by Busse (1894 p. 923).

454. Beijerinck's Malate Asparagin Solution

Constituents:

1. Water (ditch)	1000.0	cc.
2. Mohr salt $(NH_4)_2SO_4$	0.1306	g.
3. $MgSO_4$	0.4926	g.
4. NaCl	0.1	g.
5. Sodium malat	0.1	g.
6. Asparagin	0.1	g.
7. Potassium phosphate	0.2	g.
8. Na_2CO_3	1.0	g.

Preparation:
(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1 liter of ditch water ("Grabenwasser") containing 37.5 $MgSO_4$ per liter.
(2) Distribute into flasks or beaker. Cover the beaker with a glass plate or fill the flask full to reduce the surface exposed to the air.

Sterilization: Not specified.

Use: To study reduction of sulphates. The author reported the presence of H_2S and SO_3 after 3 days.

Variants:
(a) The author used the following solution to study sulphate reduction by *Spirillum tenue*. The water used was "Grabenwasser" and the medium was made alkaline by the addition of Na_2CO_3

1. Water (ditch)	1000.0	cc,

2. Sodium malat (natrium malate)	10.0 g.
3. Asparagin	5.0 g.
4. Potassium phosphate	2.5 g.
5. NaCl	2.5 g.
6. Mohr salt $(NH_4)_2SO_4$	1.0 g.

(b) Beijerinck detected the production of H_2S and SO_3 after 6 days cultivating *Spirillum desulfuricans* in the following solution:

1. Water (ditch)	1000.0 cc.
2. Gypsum ($CaSO_4$)	2.0 g.
3. Sodium malat (natrium malate)	0.5 g.
4. Asparagin	0.5 g.
5. Potassium phosphate	0.1 g.
6. Na_2CO_3	

Reference: Beijerinck (1895 pp. 55, 56, 107).

455. Zikes' Alcohol Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose (2.0%)	20.0 g.
3. Asparagin (0.25%)	2.5 g.
4. K_2HPO_4 (0.1%)	1.0 g.
5. $MgSO_4$ (0.05%)	0.5 g.
6. Alcohol	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Distribute in 10.0 cc. lots.
(3) To each 10.0 cc. lot add 5, 10, 15 or 20 drops of alcohol.

Sterilization: Not specified.

Use: To study volutin production by yeast and *Oidium lactis*.

Reference: Zikes (1922 p. 34).

456. Gosio's Glycerol Aspartic Acid Solution

Constituents:

1. Water	800.0 cc.
2. Glycerol	15.0 g.
3. NaCl	2.5 g.
4. Na_2CO_3	2.5 g.
5. K_2HPO_4	1.0 g.
6. Aspartic acid	1.5 g.
7. Grape sugar	50.0 g.
8. $CaCO_3$	20.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: To study fermentation by *Vibrio cholerae asiaticae.*

Reference: Gosio (1894 p. 25).

457. Gärtner's Glycerol Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin	1.0 g.
3. Glycerol	2.5 g.
4. Dextrose	2.5 g.
5. Potassium phosphate	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Flask in 30.0 cc. lots.

Sterilization: Not specified.

Use: To study nitrite oxidation by nitrate fungus. The author reported trace of nitrite after 4 weeks incubation.

Reference: Gärtner (1898 p. 4).

458. Borrel, de Coulon, Boez and Quimaud's Glycerol Asparagin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $FeSO_4$	0.03 g.
3. Potassium silicate	0.02 g.
4. Glycerol	20.0 g.
5. Mannitol	5.0 g.
6. Glucose	5.0 g.
7. Asparagin	4.5 g.
8. $(NH_4)_2CO_3$	1.0 g.
9. $NaNO_3$	1.0 g.
10. $MgSO_4$	0.25 g.
11. $KHSO_4$	0.25 g.
12. KH_2PO_4	0.5 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 in 1.
(2) Adjust to pH = 6.9.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

Reference: Borrel, de Coulon, Boez and Quimaud (1922 p. 389).

459. Müller, Thurgan and Osterwalder's Malate Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Malic acid	3.0 g.
3. Asparagin	10.0 g.
4. $MgSO_4$	0.4 g.
5. K_2HPO_4	2.0 g.
6. NaCl	2.0 g.
7. Levulose	40.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Cultivation of *Bacterium mannitopoeum* and bacteria from wines. There is very little growth on this medium. Organisms grow better if peptone and potassium malate be added to the medium.

Reference: Müller, Thurgan and Osterwalder (1912–13 p. 161).

460. Maassen's Glucose Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4$ (crystalline)	0.4 g.
3. Asparagin	10.0 g.
4. $CaCl_2$	0.01 g.
5. Na_2HPO_4	2.0 g.
6. $NaCO_3$ (crystalline).	2.5 g.
7. Glucose	5.0 to 10.0 g.
8. Malic acid	7.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Method not given.

Use: As a general synthetic culture medium.

Reference: Klimmer (1923 p. 172).

461. Clark and Lub's Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Na_2HPO_4 (anhydrous)	7.0 g.
or $Na_2HPO_4 \cdot 2H_2O$	8.8 g.
3. KH phthalate	2.0 g.
4. Aspartic acid	1.0 g.
5. Glucose	4.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 800.0 cc. of warm distilled water.

(2) Cool the solution and make up to a liter.

(3) The hydrogen ion concentration of the medium is fixed by the composition. It should be very close to pH 7.0, slightly red with phenol red. All materials should be recrystallized or if used from stock furnished by manufacturers, should be carefully examined. The di-sodium hydrogen phosphate may be used either as the anhydrous salt obtained by dessication in vacuo in 100°C. or else as the salt containing two molecules of water of crystallization. This is obtained by exposing the recrystallized $Na_2HPO_412H_2O$ for two weeks. Use 0.88 per cent of $Na_2HPO_42H_2O$.

Sterilization: Heat in an autoclave for 15 minutes after the pressure has reached 15 pounds, provided the total time of exposure to heat is not more than one-half hour.

Use: Differentiation of colon-aerogenes group.

References: Clark and Lubs (1915, 160), Levine (1921 p. 118), Committee American Public Health Assoc. (1925 p. 11).

462. Remy and Sugg's Citrate Asparagin Solution (Proskauer and Beck)

Constituents:

1. Water	1000.0 cc.
2. Glucose (0.1%)	1.0 g.
3. Asparagin (0.5%)	5.0 g.
4. Citric acid (0.075%)	7.5 g.
5. K_3PO_4 (0.5%)	5.0 g.
6. $MgSO_4$ (0.25%)	2.5 g.
7. K_2SO_4 (0.25%)	2.5 g.
8. NaCl (0.15%)	1.5 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: Cultivation of the tubercle bacilli.

Reference: Proskauer and Beck (1894 p. 138).

463. Uschinsky's Glycerol Asparaginate Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol	30.0–40.0 g.
3. NaCl	5.0–7.0 g.
4. $CaCl_2$	0.1 g.
5. $MgSO_4$	0.2–0.4 g.
6. K_2HPO_4	2.0–2.4 g.
7. Amm. lactate	6.0–7.0 g.
8. Sodium asparaginate	3.4 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: General culture medium. Author reported growth was as good as in bouillon. Diphtheria and tetanux toxin also produced. Glucose increases production of tetanus toxin.

Variants:

(a) The author added 10.0 to 20.0 g. glucose.

(b) Cramer specified the use of 10.0 g.

ammonium lactate, 3.4 g. asparagin, 40.0 g. glycerol, 5.0 g. NaCl, 0.2 g. $MgSO_4$, but did not specify the amount of K_2HPO_4.

(c) Vaughan specified the use of 1.0 g. NaCl, 0.4 g. K_2HPO_4 and 6.0 g. ammonium lactate.

(d) Hadley specified the use of 6.5 g. ammonium lactate, 0.32 g. asparagin, 0.3 g. $MgSO_4$, 2.5 g. K_2HPO_4 and 6.0 g. NaCl.

(e) Tanner used 3.4 g. asparagin, 10.0 g. ammonium lactate, 5.0 g. NaCl, 0.2 g. $MgSO_4 \cdot 7H_2O$, 1.0 g. K_2HPO_4 and 40.0 cc. glycerol.

(f) Bezançon and Dopter and Sacquépée used 3.0 to 4.0 g. asparagin.

(g) Harvey used 6.0 g. NaCl, 0.1 g. CaCl, 0.3 g. $MgSO_4$, 2.5 g. K_2HPO_4, 8.0 g. ammonium lactate, 3.5 sodium asparaginate, and 35.0 g. glycerol.

(h) Harvey used 3.4 g. asparagin, 10.0 g. ammonium lactate, 5.0 g. NaCl, 0.2 g. $MgSO_4$, 0.1 g. $CaCl_2$, 1.0 g. K_2HPO_4, and 40.0 g. glycerol.

(i) Harvey used 5.0 g. NaCl, 0.1 g. NaCl, 0.2 g. $MgSO_4$, 2.0 g. K_2HPO_4, 6.0 g. ammonium lactate, 3.0 g. potassium or sodium asparaginate and 30.0 g. glycerol.

(j) Roux and Rochaix used 3.0 to 4.0 g. asparagin instead of sodium asparaginate.

(k) Besson used 30.0 g. glycerol, 5.0 g. NaCl, 0.2 g. $MgSO_4$, 2.0 g. K_2HPO_4, 6.0 g. ammonium lactate and 3.0 g. asparagin.

References: Uschinsky (1893 p. 316), Cramer (1894 p. 171), Vaughan (1896 p. 312), Smith (1905 p. 197), Hadley (1907 p. 101), Roux and Rochaix (1911 p. 246), Kalb and Wasserman (1912 p. 394), Löhnis (1913 p. 44), Tanner (1919 p. 69), Bezançon (1920 p. 116), Besson (1920 p. 37), Dopter and Sacquépée (1921 p. 121), Harvey (1921–22 pp. 102, 103), Klimmer (1923 p. 394).

464. Sullivan's Glycerol Nitrate Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin	15.0 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4$	0.2 g.
5. Ammonium lactate	0.8 g.
6. NaCl	1.0 g.
7. KNO_3	0.3 g.
8. Glycerol	10.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: General culture medium. The author reported that about 40 variaties developed on this medium including chromogenic bacteria and pathogenic forms.

Variants:

(a) Hadley substituted 25.0 g. glycerol for 10.0 g.

(b) Magnusson used 5.0 g. NaCl, 0.5 g. ammonium lactate, 1.0 g. of sodium asparaginate and 0.2 g. $NaNO_3$. He used this medium to study slime and capsule production by *Bacterium lactis viscosum*, and streptococci. Slime and capsules were produced.

Reference: Sullivan (1905–06 p. 115), Hadley (1907 p. 101), Magnusson (1918 p. 467).

465. Hadley's Glycerol Asparagin Solution No. V

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium lactate	5.0 g.
3. Ammonium phosphate	10.0 g.
4. Asparagin	10.0 g.
5. $FePO_4$	0.2 g.
6. Glycerin	40.0 g.
7. $MgSO_4$	0.3 g.
8. K_2HPO_4	3.0 g.
9. NaCl	1.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

Sterilization: Not specified.

Use: Growth and toxin production of *Bacillus diphtheriae*. Author reported slight growth.

Reference: Hadley (1907 p. 101).

466. Löwenstein's Glycerol Asparagin Solution

Constituents:

1. Asparagin	6.0 g.
2. Ammonium lactate	0.0 or 6.0 g.
3. Sodium phosphate (neutral)	3.0 g.
4. NaCl	6.0 g.
5. Glycerin	40.0 g.

Preparation: (1) Original article does not specify the use of water as a solvent. The ammonium lactate may be omitted if desired.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

Reference: Löwenstein (1913 p. 591).

467. Lockemann's Citrate Glycerol Asparagin Solution (Löwenstein)

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4	5.0 g.
3. $MgSO_4$	0.6 g.
4. Magnesium citrate	2.5 g.
5. Asparagin	5.0 g.
6. Glycerol	20.0 g.
7. Soda about	2.5 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

Variants:

(a) Lockemann added 3.0 g. NaH_2PO_4 and omitted the soda.

(b) Harvey used 2.5 g. NaOH instead of the soda.

(c) Mueller used 2.6 g. magnesium citrate and adjusted the reaction to pH = 7.0 using NaOH instead of soda.

References: Löwenstein (1913 p. 592), Lockemann (1919 p. 421), Harvey (1921–22 p. 103), Mueller (1926 p. 3).

468. Frouin's Glycerol Asparagin Solution (Bezançon)

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4$	1.0 g.
3. Potassium phosphate	1.0 g.
4. Asparagin	5.0 g.
5. Lactose	6.0 g.
6. Sodium citrate	3.0 g.
7. Glycerol	40.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

Reference: Bezançon (1920 p. 548).

469. Hadley's Urea Glycocoll Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium lactate	32.5 g.
3. $CaCl_2$	0.4 g.
4. Glycerin	170.0 g.
5. Glycocoll	5.0 g.
6. $MgSO_4$	1.6 g.
7. K_2HPO_4	12.5 g.
8. NaCl	30.0 g.
9. Urea	25.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in sufficient water to make 1000.0 cc.

Sterilization: Not specified.

Use: Growth of *Bacillus diphtheriae* and study of toxin production.

Reference: Hadley (1907 p. 101).

470. Beijerinck's Urea Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4	0.25 g.
3. Asparagin	2.5 g.
4. Urea	50.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of *Planosarcina ureae; Urobacillus pasteurii* did not develop in this medium.

Reference: Beijerinck (1901 p. 53).

471. Beijerinck's Urea Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4	0.9 g.
3. $(NH_4)_2CO_3$	0.2 g.
4. Asparagin	0.25 g.
5. Urea	1.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of urea organisms from the soil, *Urococcus ureae* and *Urobacillus pasteurii*. A soil culture will change all the urea after incubation of 10 days.

Reference: Beijerinck (1901 p. 53).

472. Sears' Uric Acid Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin	10.0 g.
3. Na_2CO_3	2.5 g.
4. Na_2HPO_4	2.0 g.
5. $MgSO_4$	traces
6. $CaCl_2$	traces
7. Uric acid	0.902 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: To determine production of urea and study of nitrogen metabolism. Sears detected urea by urease method of Van Slyke. Ammonia determined by Folin's aeration method, amino acid by Van Slyke's method, total nitrogen by Kjeldahl-Gunning-Arnold method.

Reference: Sears (1916 p. 132).

473. Kappen's Cyanamide Asparagin Solution

Constituents:

1. Water	1000.0 cc.
2. Calcium cyanamide (crude).	15.0 g.
3. K_2HPO_4 (0.5)	5.0 g.
4. Asparagin (0.1%)	1.0 g.
5. Glucose (0.1%)	10.0 g.

Preparation:

(1) Grind 15.0 g. of crude calcium cyanamide to a powder and dry sterilize for 3 hours at 160°C.

(2) Dissolve (1) in sterile water.

(3) Filter thru a sterile folded filter into a sterile flask.

(4) Mix with a sterile solution containing 0.5% K_2HPO_4, 0.1% asparagin and 0.1% dextrose. The final volume should be 1000.0 cc.

(5) All the flasks, filters, funnel, etc., should be sterile and the entire process of preparation carried out under aseptic conditions.

Sterilization: Method of sterilization of solutions and apparatus not specified.

Use: To study cyanamide decomposition by bacteria.

Variants: The author suggested the following solution:

1. Water	1000.0 cc.
2. Asparagin (0.1%)	1.0 g.
3. K_2HPO_4 (0.5%)	5.0 g.
4. Glucose (1.0%)	10.0 g.
5. Cyanide (0.3%)	3.0 g.

Reference: Kappen (1909 pp. 385, 391).

474. Davis and Ferry's Basal Cystine Tryptophane Solution

Constituents:

1. Water	1000.0 cc.
2. Tryptophane	0.3 g.
3. Cystine	0.4 g.

Preparation:

(1) Add 2 and 3 to 1.

(2) Add one of the combinations given under added nutrients to (1) and heat in flowing steam for 15 minutes.

(3) Add enough N/10 NaOH to give final reaction pH = 8.0 to 8.2.

(4) Steam again for 15 minutes and check reaction colorimetrically (using phenolsulphonphthalein and standardized H_3BO_3-KCl-NaOH solutions.

(5) Distribute as desired.

Sterilization: Sterilize for 20 minutes at 115°C.

Use: Cultivation of *Bact. diphtheriae* for production of toxin. Authors report better toxin production if 10.0% bouillon be added. All amino acids prepared specially, in pure form. Cultures must become accustomed to medium by adding small portions of it to bouillon.

Added nutrients and variants: The authors added the following compounds to the basic solution:

(a)

tyrosine	1.0 g.
leucine	3.0 g.
glutaminic acid hydrochloride.	1.6 g.
glycocoll	0.4 g.
creatin	0.1 g.
creatinin	0.1 g.
sodium asparaginate	1.4 g.
NaCl	4.0 g.
K_2HPO_4	3.0 g.

(b) Used 4.0 g. tryptophane in the basal solution and added the following:

tyrosine	2.5 g.
leucine	2.5 g.
glutaminic acid hydrochloride.	1.9 g.
glycocoll	0.85 g.
creatin	0.1 g.
creatinin	0.1 g.
sodium asparaginate	1.2 g.
histidine dichloride	0.3 g.
NaCl	4.0 g.
K_2HPO_4	3.0 g.
$MgSO_4$	0.5 g.
KNO_3	0.2 g.

(c) Used 0.6 g. tryptophane in the basal solution and added:

tyrosine	0.8 g.
leucine	3.0 g.
glutaminic acid hydrochloride	2.5 g.
creatin	0.2 g.
glycocoll	0.8 g.
histidine dichloride	0.5 g.
glucosamine hydrochloride	1.5 g.

sodium asparaginate	0.5 g.
K_2HPO_4	3.0 g.
$MgSO_4$	0.4 g.
KNO_3	0.2 g.

(d) Used 1.25 g. tryptophane in the basal solution and added:

histidine dichloride	1.25 g.
glucosamine hydrochloride	2.75 g.
NaCl	2.50 g.
K_2HPO_4	3.0 g.
$MgSO_4$	0.4 g.

(e) Used 0.6 g. tryptophane in the basal solution and added:

tyrosine	1.25 g.
leucin	3.00 g.
glutaminic acid hydrochloride	2.50 g.
glycocoll	0.85 g.
sodium asparaginate	1.00 g.
histidin dichloride	0.5 g.
creatin	0.2 g.
creatinin	0.08 g.
xanthin	0.05 g.
hypoxanthin	0.05 g.
NaCl	4.0 g.
K_2HPO_4	3.0 g.
$MgSO_4$	0.50 g.
glucose	1.5 g.
KNO_3	0.20 g.

(f) Used 1.6 g. tryptophane in the basal solution and added:

glutaminic acid hydrochloride	2.5 g.
glucosamine hydrochloride	1.5 g.
NaCl	2.5 g.
K_2HPO_4	3.0 g.
$MgSO_4$	0.4 g.

(g) Used 1.0 g. tryptophane in the basal solution and added:

NaCl	2.50 g.
K_2HPO_4	3.00 g.
$MgSO_4$	0.4 g.

(h) Used 1.6 g. tryptophane in the basal solution and added:

glutaminic acid hydrochloride	2.5 g.
glucosamine hydrochloride	1.0 g.
histidine dichloride	1.20 g.
NaCl	2.5 g.
K_2HPO_4	3.0 g.
$MgSO_4$	0.4 g.

Reference: Davis and Ferry (1919 pp. 226, 227, 228, 229, 230).

475. Berthelot's Amino Acid Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	2.0 g.
3. $MgSO_4$	1.0 g.
4. Glycocoll	1.5 g.
5. Sodium asparaginate	1.0 g.
6. Glutamic hydrochloride	1.0 g.
7. Alanine	1.0 g.
8. Histidine chlorohydrate	1.0 g.
9. Tyrosine	0.5 g.
10. Tryptophane	0.5 g.
11. Leucine	0.6 g.
12. Phenylalanine	0.6 g.
13. Guanidine hydrochloride	0.4 g.
14. Creatine	0.4 g.
15. Glucosamine hydrochloride	0.4 g.
16. Cystine	0.2 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16 in 1.

(2) Adjustment of reaction not specified.

Sterilization: Sterilize with ether (method not given).

Use: Toxin production by *Proteus vulgaris*. This medium will not permit successive growth of the organism.

Reference: Berthelot (1914 p. 917).

476. Armand-Delille, et al, Glycocoll Arginine Solution (Synthetic Medium 164)

Constituents:

1. Water	1000.0 cc.
2. NaCl	5.0 g.
3. KH_2PO_4	5.0 g.
4. Magnesium citrate	2.4 g.
5. Glucose	4.0 g.
6. Glycerol	40.0 g.
7. Glycocoll	4.0 g.
8. Arginine	2.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

(2) Add 4.0 cc. of N/100 NaOH to (1) after sterilization.

Sterilization: Method not given.

Use: Cultivation of Koch's bacilli. Medium gives good growth.

References: Armand-Delille, Mayer, Schaeffer and Terroine (1913 p. 274), Harvey (1921–22 p. 104).

477. Armand-Delille, et al, Synthetic Solution 104

Constituents:

1. Water	20.0 cc.
2. NaCl	1.25 g.
3. Magnesium citrate	0.6 g.

4. NaH_2PO_4	1.25 g.
5. Glycocoll	0.50 g.
6. Aspartic acid	0.5 g.
7. Carnosine nitrate	0.1 g.
8. Creatinin	0.1 g.
9. Sarcosine	0.1 g.
10. Glucose	0.5 g.
11. Inosite	0.1 g.
12. Glycerol	10.0 g.

(Water given as 20.0 cc. In similar media 389 and 391 water given as 250.0 cc.)

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 in 1.

(2) Add 1.0 cc. of N/100 NaOH to (1) after sterilization.

Sterilization: Method not given.

Use: Cultivation of Koch's bacilli.

Reference: Armand-Delille, Mayer, Schaeffer and Terroine (1913 p. 273).

478. Armand-Delille, et al, Glycocoll Aspartic Acid Solution (Synthetic Medium 118)

Constituents:

1. Water	1000.0 cc.
2. NaCl	5.0 g.
3. Magnesium citrate	2.4 g.
4. NaH_2PO_4	5.0 g.
5. Glycocoll	2.0 g.
6. Aspartic acid	2.0 g.
7. Glucose	2.0 g.
8. Scombrine sulphate	2.0 g.
9. Glycerol	40.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

(2) Add 4.0 cc. of N/100 NaOH to (1) after sterilization.

Sterilization: Method not given.

Use: Cultivation of Koch's bacilli.

References: Armand-Delille, Mayer, Schaeffer and Terroine (1913 p. 273).

479. Zipfel's Tryptophane Asparagin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Asparagin	
3. Ammonium lactate	5.0 g.
4. K_2HPO_4	2.0 g.
5. $MgSO_4$	0.2 g.
6. Tryptophane (0.1%)	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1 (amount of asparagin not specified).

(2) Adjustment of reaction not specified.

(3) Tube in 10.0 cc. lots.

Sterilization: Method not given.

Use: Study of indol production.

Variants:

(a) Zipfel used 0.3 or 0.5% tryptophane instead of 0.1%.

(b) Barthel used 5.0 g. asparagin and 0.3 g. Tryptophane.

(c) Barthel used 5.0 g. asparagin, 0.3 g. tryptophane and omitted the 5.0 g. ammonium lactate.

References: Zipfel (1912 p. 74), Barthel (1921 p. 85).

SUBGROUP I-C. SECTION 6

Liquid media, or basal solutions with constituents of known chemical composition containing organic nitrogen other than amino acids; amino acids not present.

A_1.* Organic nitrogen supplied as cyanogen or its compounds.

B_1. Nitrogen supplied as cyanamide.
  Kappen's Glucose Cyanamide Solution 480
  Vierling's Glucose Cyanamide Solution 481

B_2. Nitrogen supplied as dicyanamide.
  Perotti's Dicyandiamide Solution ... 482
  Perotti's Glucose Dicyandiamide Solution 483
  Kappen's Glucose Dicyandiamide Solution 484

B_3. Nitrogen supplied as ferrocyanamide.
  Doryland's Basal Ferrocyanide Salt Solution 485
  Bokorny's Sucrose Ferrocyanide Solution 486

A_2.* Organic nitrogen supplied as urea.

B_1. Incomplete or basal solutions, employed with additional nutrients.
  Stutzer and Hartleb's Basal Urea Solution 487
  Beijerinck's Basal Urea Salt Solution. 488
  Waksman's Basal Urea Salt Solution 489
  Christensen's Basal Urea Salt Solution 490

B_2. Complete media.

C_1. Containing no additional organic compounds.

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*See also A_3, A_4, A_5 and A_6 on page 136.

Fremlin's Urea Solution........... 491
Wherry's Urea Solution............ 492
Evans' Urea Solution.............. 493
Percival's Urea Solution........... 494

C_2. Containing additional organic compounds.

D_1. Carbohydrates added.
Murray's Glucose Urea Solution.... 495
Vierling's Glucose Urea Solution.... 496
Thomas' Sucrose Urea Solution..... 497
Hugounenq and Doyon's Sucrose Urea Solution.................. 498

D_2. Alcohols added.
Hadley's Glycerol Urea Solution.... 499
Harvey's Glycerol Urea Solution.... 500

A_3. Organic nitrogen supplied as uric acid.
Koser's Glycerol Uric Acid Solution.. 501
Löhnis' Uric Acid Solution.......... 502

A_4. Organic nitrogen supplied as amines and amides.
Bokorny's Amine Solution.......... 503
Omeliansky's Amine Solution....... 504
Beijerinck and Minkman's Glucose Amine Solution.................. 505

A_5. Organic nitrogen supplied as hippuric acid.
Went's Basal Hippuric Acid Solution. 506
Stapp's Hippurate Solution......... 507
Löhnis' Hippurate Solution......... 508

A_6. Organic nitrogen supplied other than A_1 to A_5.
Stoklasa's Glucose Lecithin Solution. 509
Stoklasa's Nucleic Acid Solution.... 510
Went's Basal Creatin Solution...... 511
Stutzer's Glucose Creatin Solution.. 512
Meyers' Taurine Solution........... 513
Wherry's Theobromin Solution...... 514
Bokorny's Picric Acid Solution...... 515

480. Kappen's Glucose Cyanamide Solution

Constituents:

1.	Water	1000.0 cc.
2.	Glucose (1.0%)	10.0 g.
3.	KH_2PO_4 (0.5%)	5.0 g.
4.	Cyanamide (0.5%)	5.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: To study decomposition of cyanamide by *Penicillium brevicaule*, *Stysanus stemonitis* and *Cladosporium*. Cyanamide in 2.0% strength was toxic for some of the types studied. Using 0.5% cyanamide and varying the dextrose content, the most NH_3 was produced from the cyanamide with 0.5 to 1.0% dextrose.

Variants:

(a) The author used 1.0 or 2.0% cyanamide instead of 0.5%.

(b) The author used 0.1, 0.5 or 1.0% glucose with 0.5% cyanamide.

Reference: Kappen (1910 p. 635).

481. Vierling's Glucose Cyanamide Solution

Constituents:

1.	Water	1000.0 cc.
2.	K_2HPO_4	1.0 g.
3.	$CaCl_2$	0.1 g.
4.	$MgSO_4$	0.1 g.
5.	$FeCl_3$	trace
6.	NaCl	trace
7.	Calcium cyanamide	0.2 g.
8.	Glucose	5.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Cultivation of mycobacteria.

Reference: Vierling (1920 p. 202).

482. Perotti's Dicyandiamide Solution

Constituents:

1.	Water	1000.0 cc.
2.	K_2HPO_4	0.5 g.
3.	NaCl	0.5 g.
4.	$CaSO_4$	0.5 g.
5.	$MgSO_4$	0.5 g.
6.	Iron chloride	drops
7.	$C_2N_4H_4$ (dicyandiamide)	0.5 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Distribute in flasks.

Sterilization: Sterilize on 3 successive days at 120°C. for 10 minutes, each day.

Use: To study influence of dicyandiamide on field plants. Author reported that the nitrogen content was increased. As a control KNO_3 was used instead of dicyandiamide.

Reference: Perotti (1908 p. 225).

483. Perotti's Glucose Dicyandiamide Solution

Constituents:

1.	Water	1000.0 cc.
2.	dicyandiamide	2.0 g.
3.	Dextrose	0.1 g.
4.	K_2HPO_4	0.5 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1. (Use tap water or distilled water, with traces of NaCl, Na_2SO_3 and $CaCl_2$.)

(2) Distribute 200.0 cc. of 1 in a one liter flask.

(3) Inoculate with 0.2 g. of garden earth.

Sterilization: Not specified.

Use: Cultivation of dicyandiamide bacteria from the soil.

Variants: The author gives the following solution:

1. Water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.3 g.
5. NaCl	0.1 g.
6. Iron chloride	0.01 g.
7. Glucose (0.5%)	5.0 g.
8. Dicyandiamide 0.1, 1.0, 5.0 or 10.0%.	

Reference: Perotti (1908, vol. 21, p. 207), (1908, vol. 20, p. 516).

484. Kappen's Glucose Dicyanamide Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4 (0.05%)	5.0 g.
3. $MgSO_4$ (0.5%)	5.0 g.
4. Dicyanamide (1.0%)	10.0 g.
5. Glucose (10.0%)	100.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: To study decomposition of cyanamide by *Penicillium brevicaule*, *Stysanus stemonitis* and *Cladosporium*.

Reference: Kappen (1910 p. 637).

485. Doryland's Basal Ferrocyanide Salt Solution

Constituents:

1. Distilled water	500.0 cc.
2. HCl	dilute
3. $MgSO_4$	0.5 g.
4. CaO	0.01 g.
5. $Fe_2(SO_4)_3$	0.01 g.
6. $MgSO_4$	0.01 g.
7. $K_6Fe_2(CN)_{12}$, amount not given.	
8. H_2SO_4	
9. H_3PO_4	
10. N/0.2578 NaOH	
11. N/0.6205 KOH	

Preparation:

(1) Dilute the HCl so that 1.0 cc. is not quite neutralized by 1.0 cc. of silicate solution made by dissolving 24.0 g. K_2SiO_3 and 8.4 g. Na_2SiO_3 in 500.0 cc. distilled water, using phenolphthalein as indicator.

(2) Add to HCl the following salts:

$MgSO_4$	0.5 g.
$Fe_2(SO_4)_3$	0.01 g.
CaO	0.01 g.
$MnSO_4$	0.01 g.
$K_6Fe_2(CN)_{12}$, amount not given.	

(3) Standardize (2) against silicate solution so that 1.0 cc. is equivalent to 1.0 cc. using methyl orange as indicator.

(4) Standardize a solution of H_2SO_4 in same way as HCl omitting salts.

(5) Standardize H_3PO_4 in similar manner as HCl omitting the salts and using phenolphthalein as indicator.

(6) Mix the acids in the following ratio:

HCl	153.5 cc.
H_2SO_4	77.0 cc.
H_3PO_4	116.0 cc.

(7) Mix equal quantities of N/0.6205 KOH and N/0.2578 NaOH.

(8) 1.0 cc. of (7) should neutralize 1.0 cc. of (6), phenolphthalein as indicator.

(9) Draw acid and base solution into separate burettes and allow to stand several hours to sterilize.

Sterilization: Method specified in preparation.

Use: Synthetic culture medium.

Added nutrients: Acetic acid or glucose were used as additional carbon source. When using acetic acid mix with H_3PO_4 in step (5). When using glucose add a sufficient quantity of a sterile aqueous glucose solution to a mixture of equal parts of (6) and (7) so that there will be 10.0 g. of glucose per liter of solution.

Reference: Doryland (1916 p. 148).

486. Bokorny's Sucrose Ferrocyanide Solution

Constituents:

1. Water	1000.0 cc.
2. Sucrose	30.0 g.
3. Potassium ferrocyanide ($K_4Fe(CN)_6$)	6.0 g.
4. K_2HPO_4	1.0 g.
5. $MgSO_4$	0.32 g.
6. $CaCl_2$	0.08 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of *Schizomycetes*. Author reported that mold spores did not develop in this medium. *Schizomycetes* developed producing lactic acid. The acid produced may decompose the $K_4Fe(CN)_6$. Potassium ferrocyanide was a suitable nitrogen source for schizomycetes but not for molds.

Variants: Bokorny also used the following solution:

1. Water	1000.0	cc.
2. Sucrose	20.0	g.
3. Potassium ferrocyanide	2.0	g.
4. K_2HPO_4	2.0	g.
5. $MgSO_4$	0.052	g.
6. $CaCl_2$	0.012	g.

Reference: Bokorny (1917 pp. 343, 344).

487. Stutzer and Hartleb's Basal Urea Solution

Constituents:

1. Water	1000.0	cc.
2. Urea	20.0	g.
3. Glycerin	10.0	g.
4. Potassium phosphate	1.0	g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of the organisms from cases of foot and mouth disease.

Added nutrients and variants:

(a) The authors used the basic solution as a medium.

(b) Söhngen used 5.0% urea and 0.025 K_2HPO_4 as the basic solution and added 1.0% of one of the following:
ammonium oxalate
sodium acetate
Rochelle salt
ammonium citrate
ammonium malate
He used the media to study urea decomposition by bacteria from the soil.

(c) Söhngen used 0.05% K_2HPO_4 and 2.0% urea in the basic solution and added 5.0 to 61.5 mg. of nitrogen in the form of asparagin or ammonium malate to study urea decomposition by *Urobacillus Jakschii*.

(d) Söhngen used 0.05% K_2HPO_4 and 5.0% urea in the basic solution and added 5.0 to 61.5 mg. of nitrogen in the form of asparagin or ammonium malate to study urea decomposition by *Bacillus erythrogenes*.

(e) Söhngen used 0.05% K_2HPO_4 and 3.0% urea in the basic solution and added 1.0% of calcium malate or calcium citrate to study urea decomposition by bacteria from the soil.

(f) Söhngen used a 0.5% urea and 0.05% K_2HPO_4 solution without additions to cultivate *Bacillus erythrogenes*.

(g) Percival used 5.0% urea and 0.25% K_2HPO_4 in the basic solution and added 1.0% $(NH_4)_2SO_4$ to enrich urea splitting organisms found in the soil and in urine.

(h) Giltner used 5.0% urea and 0.05% K_2HPO_4 in the basic solution and added 0.5 to 1.0% of one of the following:
ammonium malate
calcium malate
calcium citrate
ammonium citrate
He used the media to show urea decomposition.

References: Stutzer and Hartleb (1897 p. 404), Söhngen (1909 pp. 91, 93, 94, 95), Percival (1920 p. 225), Giltner (1921 p. 314).

488. Beijerinck's Basal Urea Salt Solution

Constituents:

1. Water	1000.0	cc.
2. Urea	5.0	g.
3. KH_2PO_4	0.025	g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add 1.0% of one of the added nutrients.

Sterilization: Not specified.

Use: Cultivation of urea organisms from the soil, *Urobacillus pasteurii*, *Urococcus ureae*. Author reported that about 2.0% of the urea was changed by a soil culture using oxalate as carbon source, 2.0% using sodium acetate, 2.0% using Seignette salt, 3.0% using ammonium citrate and 4.% using ammonium malate.

Added nutrients and variants:

(a) 1.0% of one of the following carbon sources were added:

ammonium oxalate	1.0 g.
sodium acetate	
Seignette salt	
(potassium sodium tartrate)	1.0 g.
Ammonium citrate	1.0 g.
Ammonium malate	1.0 g.

(b) Löhnis used 50.0 g. urea and 0.5 g. K_2HPO_4 in the basic solution, and added 0.5 to 1.0% ammonium or calcium malate, or calcium citrate or tartrate.

(c) Percival used 2.5% KH_2PO_4 and 5.0% urea and added 1.0% sodium acetate.

References: Beijerinck (1901 p. 37), Löhnis (1913 p. 108), Percival (1920 p. 225).

489. Waksman's Basal Urea Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol	30.0 g.
3. K_2HPO_4	1.0 g.
4. KCl	0.5 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.01 g.
7. Urea	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Add 2.0 g. of one of the added nutrients.

(3) Tube in 10 to 12 cc. quantities.

Sterilization: Sterilize at 15 pounds for 15 minutes.

Use: To study metabolism of *Actinomycetes.*

Added nutrients: Waksman added 2.0 g. of one of the following nitrogen sources:

$NaNO_3$ $(NH_4)_2SO_4$

$NaNO_2$ $(NH_4)_2CO_3$

Reference: Waksman (1920 p. 3).

490. Christensen's Basal Urea Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Urea	20.0 g.
3. K_2HPO_4 (1.0% soln.)	20.0 cc.
4. $CaCO_3$	
5. $MgSO_4$	small amount
6. $FeSO_4$	small amount

Preparation:

(1) Prepare a 1.0% solution of K_2HPO_4.

(2) Add 2.0 g. of urea (Kahlbaum's I), 2.0 cc. of (1), some $CaCO_3$, and very small quantities of $MgSO_4$ and $FeSO_4$ (amount not specified) to 1000.0 cc. distilled water.

(3) Add one of the added nutrients.

(4) Distribute into 450.0 cc. Erlenmeyer flasks.

(5) Inoculate with a little soil infusion.

Sterilization: Not specified.

Use: To study urea decomposition by soil forms. Ammonia formation was apparent after 14 days when humus was added and after 5 days with glucose. If humus or glucose were not added, there was apparently no ammonia formation.

Added nutrients: One of the following materials were added:

(a) Humus acid small amount. Add sufficient dilute NaOH to keep the humus acid in solution.

(b) Potassium salt of humus acid 2.0%.

(c) Glucose 1.0%.

(d) "Buchenrohhumas" 0.1%.

(e) Xylose 1.0%.

(f) Sodium formate 0.2%.

(g) Meat extract 2.0%.

(h) Potassium humate 0.1% (see (8) below).

(i) Humin 0.2% (see (10) below).

Materials in (h) and (i) prepared as follows:

(1) Dissolve 300.0 g. saccharose in 420.0 cc. water.

(2) Add 15.0 g. concentrated H_2SO_4 to (1).

(3) Place on a boiling water bath for 7 or 8 hours, adding the water lost by evaporation.

(4) After completing the heating a voluminous sediment forms at the bottom.

(5) Filter and carefully wash.

(6) Treat the precipitate with an excess of 5.0% KOH solution. A portion of the humus acid is dissolved. The humin does not dissolve.

(7) Filter.

(8) Utilize the filtrate to prepare a neutral potassium humate (method not given).

(9) Wash the precipitate to remove all traces of humus acid.

(10) Dry (9) and pulverize humin.

Reference: Christensen (1910 pp. 340, 348).

491. Fremlin's Urea Solution

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2SO_4$	1.0 g.
3. Potassium phosphate	1.0 g.

4. $MgCO_3$	10.0 g.
5. Urea	0.1 to 1.0 g.

Preparation:

(1) Dissolve 2 and 3 in part of 1.
(2) Dissolve 4 in remainder of 1.
(3) After sterilizing (1) and (2) mix under aseptic conditions.
(4) Add various quantities (0.1 to 1.0 g.) sterile urea.

Sterilization: Method not specified.

Use: To study nitrogen oxidation by *Nitrosobacter*.

Reference: Fremlin (1903 p. 369).

492. Wherry's Urea Solution

Constituents:

1. Redistilled water	1000.0 cc.
2. Na_2CO_3	1.0 g.
3. KCl	1.0 g.
4. $MgSO_4$	1.0 g.
5. $CaCl_2$	1.0 g.
6. Urea	2.0 g.
7. KH_2PO_4	2.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Adjustment of reaction not given.

Sterilization: Sterilize in autoclave, time not given.

Use: To study acid proofness of *B. tuberculosis*. Author reported that after the sixth day all rods were non-acid-fast.

Reference: Wherry (1913 p. 151).

493. Evans' Urea Solution

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4$	1.0 g.
3. K_2HPO_4	2.0 g.
4. Urea	

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) No adjustment of reaction given.
(3) Tube.
(4) After sterilization add a small piece of urea to each tube.

Sterilization: Method of sterilization of the solution not specified. Sterilize a piece of solid urea by heating for ½ hour in a toluene oven at 105°C.

Use: To determine decomposition of urea.

Reference: Evans (1916 p. 445).

494. Percival's Urea Solution

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2SO_4$	2.0 g.
3. K_2HPO_4	1.0 g.
4. NaCl	2.0 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.4 g.
7. $MgCO_3$	10.0 g.
8. Urea (0.01%)	0.1 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Distribute in 50.0 cc. into conical flasks.
(3) Add 0.5 g. of basic $MgCO_3$ to each flask.
(4) To each flask add 0.01% urea.

Sterilization: Not specified.

Use: To study nitrification by soil forms.

Variants: Percival added 0.03, 0.3, 1.0 or 3.0% urea to each flask.

Reference: Percival (1920 p. 144).

495. Murray's Glucose Urea Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose	100.0 g.
3. K_2HPO_4	50.0 g.
4. $MgSO_4$	0.5 g.
5. Urea	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Tube.

Sterilization: Not specified.

Use: To study ammonification under either anaerobic or aerobic conditions.

Reference: Murray (1916 p. 602).

496. Vierling's Glucose Urea Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1 0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.1 g.
5. $FeCl_3$	trace
6. NaCl	trace
7. Glucose	10.0 g.
8. Urea	10.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: Cultivation of mycobacteria.

Reference: Vierling (1920 p. 206).

497. Thomas' Sucrose Urea Solution

Constituents:

1. Water	1000.0 cc.
2. Sucrose	200.0 g.

3. KH_2PO_4	2.25 g.
4. $MgSO_4$	0.45 g.
5. Urea	1.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Prepare a 20.0% urea solution in water.

(3) Add 20.0 cc. of sterile (2) to cool sterile (1) under aseptic conditions.

Sterilization: Sterilize (1) at 120°C. Sterilize (2) by filtration.

Use: Cultivation of yeast.

Reference: Thomas (1919 p. 800).

498. Hugounenq and Doyon's Sucrose Urea Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol	45.0 g.
3. NaCl	7.0 g.
4. Ammonium lactate	10.0 g.
5. $CaCl_2$	0.1 g.
6. $MgSO_4$	0.2 g.
7. Potassium biphosphate	2.0 g.
8. Uric acid	0.02 g.
9. Urea	5.0 g.
10. Sucrose	5.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8, 9 and 10 in 1.

Sterilization: Not specified.

Use: General culture medium. Reported as not being a suitable medium for diphtheria bacilli.

Reference: Hugounenq and Doyon (1896 p. 401).

499. Hadley's Glycerol Urea Solution

Constituents:

1. Distilled water	100.0 cc.
2. Ammonium lactate	3.2 g.
3. $CaCl_2$	0.05 g.
4. Glycerol	15.0 g.
5. $MgSO_4$	0.15 g.
6. K_2HPO_4	1.25 g.
7. NaCl	3.0 g.
8. Urea	1.6 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in sufficient water to make 100.0 cc.

Sterilization: Not specified.

Use: Growth of *Bacillus diphtheriae* and production of toxin.

Reference: Hadley (1907 p. 101).

500. Harvey's Glycerol Urea Solution

Constituents:

1. Distilled water	1000.0 cc.
2. NaCl	0.02 g.
3. $MgSO_4$	0.02 g.
4. K_2HPO_4	0.5 g.
5. $FeCl_3$	trace
6. Glycerol	10.0 g.
7. Urea	1.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not given.

Use: General culture medium. As other sources of carbon, saccharose, glucose, mannite, calcium lactate, etc., were used. As other sources of nitrogen, sodium hippurate, uric acid, asparagin, ammonium sulphate, ammonium acetate, ammonium butyrate, and ammonium lactate were used.

Reference: Harvey (1921–22 p. 104).

501. Koser's Glycerol Uric Acid Solution

Constituents:

1. Distilled water (NH_3 free)	1000.0 cc.
2. NaCl	5.0 g.
3. $MgSO_4$	0.2 g.
4. $CaCl_2$	0.1 g.
5. K_2HPO_4	1.0 g.
6. Glycerol	30.1 g.
7. Uric acid	0.5 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Adjustment of reaction not given.

(3) Tube.

Sterilization: Tube and sterilize at 13 to 15 pounds extra pressure for 15 minutes. A slight turbidity appears after autoclaving but upon cooling the solution becomes clear.

Use: *B. aerogenes* soon produces a dense clouding. *B. coli* fails to develop. Care must be taken to keep the medium ammonia free.

Variants: Ecker and Morris used 0.2 g. uric acid instead of 0.5 g.

References: Koser (1918 p. 377), Levine (1920 p. 118), Harvey (1921–22 p. 104), Ecker and Morris (1924 p. 480), Com. American Public Health Asso. (1925 p. 112).

502. Löhnis' Uric Acid Solution

Constituents:

1. Water (tap)	1000.0 cc.
2. Uric acid	3.0 g.
3. K_2HPO_4	0.5 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Distribute in small Erlenmeyer flasks.

Sterilization: Not specified.

Use: Cultivation of *B. fluorescens* and uric acid bacteria.

Reference: Löhnis (1913 p. 96).

503. Bokorny's Amine Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	2.5 g.
3. $MgSO_4$	0.2 g.
4. $CaCl_2$	0.05 g.
5. Methyl amine hydrochloride (0.5%)	5.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: To study the constituents essential for growth of molds. Molds grew readily on this medium.

Variants: The author used the following amines and amides instead of methyl amine hydrochloride:

(a) 1.0% propyl amine hydrochloride. Certain molds developed on this medium.

(b) 0.5% oxamide. Yeast did not grow in this medium.

(c) The salt concentration was changed and materials added. The medium used had the following composition:

1. Water	1000.0 cc.
2. Trimethyl amine acetic acid	5.0 g.
3. Sucrose	50.0 g.
4. K_2HPO_4	2.0 g.
5. $MgSO_4$	0.2 g.
6. $CaCl_2$	0.2 g.
7. H_3PO_4	0.0 or 1.0%

Trimethyl amine served as a nitrogen source for bacteria and without the H_3PO_4 Schizomycetes developed then the molds. In the presence of H_3PO_4 molds developed at once.

Reference: Bokorny (1917 pp. 337, 338).

504. Omeliansky's Amine Solution

Constituents:

1. Water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $MgSO_4$	0.5 g.
4. NaCl	2.0 g.
5. Methyl amine	0.5 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Adjustment of reaction not given.

(3) Add the usual amount of sterile $MgCO_3$ to the sterile fluid.

Sterilization: Sterilize by heating at 115°C. for 30 minutes.

Use: To study nitrification by nitrite formers. The methyl amine or dimethyl amine was not oxidized.

Variants: The author used 0.5 g. dimethylamine instead of methyl amine.

Reference: Omeliansky (1899 p. 485).

505. Beijerinck and Minkman's Glucose Amine Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose amine (0.5%)	5.0 g.
3. KNO_3	10.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: To study denitrification by soil forms, *Bacillus nitroxus*.

Reference: Beijerinck and Minkman (1910 p. 37).

506. Went's Basal Hippuric Acid Solution

Constituents:

1. Water	1000.0 cc.
2. Hippuric acid (0.66%)	6.6 g.

Preparation:

(1) Prepare a 0.66% solution of hippuric acid.

(2) Prepare a 5.0% solution of one of the added nutrients.

(3) Mix equal volumes of (1) and (2).

Sterilization: Not specified.

Use: Cultivation of *Monilia sitophila (Mont.) Sacc.* Maltose, glucose, lactose, sucrose and glycerol was the order of vigor of growth for the carbon sources studied.

Added nutrients: One of the following carbon sources was employed:

maltose
lactose
sucrose
glucose
glycerol

Variants: No additional carbon source was added. No growth occurred.

Reference: Went (1901 p. 593).

507. Stapp's Hippurate Solution

Constituents:

1. Water	1000.0	cc.
2. KH_2PO_4	1.25	g.
3. $MgSO_4$	0.625	g.
4. Sodium hippurate	3.1	g.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Distribute in 50.0 cc. lots in 200.0 cc. Erlenmeyer flasks.

Sterilization: Not specified.

Use: Isolation of uric acid splitting bacteria from feces and soil. *Bac. cobayae, Bac. capri, Bact., guano, Bac. musculi, Bac. hollandicus.*

Reference: Stapp (1920 p. 4).

508. Löhnis' Hippurate Solution

Constituents:

1. Water (tap)	1000.0	cc.
2. Sodium hippurate	3.0	g.
3. K_2HPO_4	0.5	g.

Preparation:
(1) Dissolve 2 and 3 in 1.
(2) Distribute in small Erlenmeyer flasks.

Sterilization: Not specified.

Use: Cultivation of hippuric acid bacteria. Author reported that fermentation took place rather slowly when the medium was inoculated with manure.

Reference: Löhnis (1913 p. 96).

509. Stoklasa's Glucose Lecithin Solution

Constituents:

1. Distilled water	8000.0	cc.
2. NH_4NO_3	20.0	g.
3. K_2SO_4	8.0	g.
4. $MgCl_2$	4.0	g.
5. NaCl	1.0	g.
6. $Al_2(SO_4)_3 + FeSO_4$	0.12	g.
7. d-glucose	80.0	g.
8. lecithin	8.0	g.

Preparation:
(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Distribute into large 2 liter Erlenmeyer flasks in 250.0 cc. lots.
(3) Adjustment of reaction not specified.
(4) After sterilization add 0.25 g. lecithin to each flask. Heat at 45 to 50° for 20 hours.

Sterilization: Sterilize thoroughly (method not given).

Use: To study the cycle of the phosphate ion in the soil. Author used *Bacillus mycoides, Bacillus subtilis, Bacillus proteus vulgaris.* Amount of phosphoric anhydride formed by each organism was about the same after 60 days. If the NH_4NO_3 be omitted the nitrogen content is too low to give good growth.

Variants: Author omitted the NH_4NO_3 and used only 0.8 g. NaCl instead of 1.0 g.

Reference: Stoklasa (1911 pp. 423, 424).

510. Stoklasa's Nucleic Acid Solution

Constituents:

1. Distilled water	8000.0	cc.
2. K_2SO_4	8.0	g.
3. $MgCl_2$	4.0	g.
4. NaCl	1.0	g.
5. $Al_2(SO_4)_3 + FeSO_4$	0.12	g.
6. Nuclein acid	8.0	g.
7. Glucose	80.0	g.

Preparation:
(1) Prepare nucleic acid from yeast by Herlant's method (method not given).
(2) Dissolve 2, 3, 4 and 5 in 1.
(3) Distribute in 250.0 cc. lots in large Erlenmeyer flasks.
(4) Add 0.25 g. nucleic acid and 2.5 g. d-glucose to each flask.
(5) Heat at 45 to 50°C. for 20 hours.

Sterilization: Method not given.

Use: To study the cycle of the phosphate ion in the soil. Author used *Bacillus mycoides, Bacillus subtilis, Bacillus proteus vulgaris. Bacillus mycoides* utilized the largest amount of phosphorous.

Reference: Stoklasa (1911 p. 428).

511. Went's Basal Creatin Solution

Constituents:

1. Water	1000.0	cc.
2. Creatin (0.66%)	6.6	g.

Preparation:
(1) Prepare a 0.66% creatin solution.
(2) Prepare a 5.0% solution of one of the added carbohydrates.
(3) Mix equal volumes of (1) and (2).

Sterilization: Not specified.

Use: Cultivation of *Monilia sitophila (Mont.) Sacc.* Maltose, glucose, lactose, sucrose and glycerol was the order of vigor of growth for the carbon sources studied.

Added nutrients: One of the following carbon sources was employed:

maltose
glucose
lactose
sucrose
glycerol

Variants: The author used the basic solution without any added materials.

Reference: Went (1901 p. 593).

512. Stutzer's Glucose Creatin Solution

Constituents:

1. Water	1000.0 cc.
2. Creatin	2.5 g.
3. K_2HPO_4	1.0 g.
4. KNO_3	3.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Reaction to be slightly alkaline.
(3) Distribute in 10.0 cc. lots.

Sterilization: Method not given.

Use: To study denitrification by *B. agilis*, *B. nitrovorus*, *B. Stutzeri*, *B. Hartlebi*. Nitrate is not reduced.

Variants: The author added 10.0 g. glucose.

Reference: Stutzer (1901 p. 83).

513. Myers' Taurine Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Taurine	5.0 g.

Preparation:

(1) Prepare taurine according to Hawk (1918).
(2) Dissolve 2 in 1.
(3) No adjustment of reaction specified.
(4) Suspend a sterile piece of filter paper, that has been soaked in 10.0% solution of lead acetate, in each flask.

Sterilization: Filter thru a Berkefeld filter.

Use: To study H_2S production.

Variants: Myers added 10.0 g. glucose.

Reference: Myers (1920 p. 249).

514. Wherry's Theobromin Solution

Constituents:

1. Redistilled water	1000.0 cc.
2. Na_2CO_3	1.0 g.
3. $CaCl_2$	1.0 g.
4. KCl	1.0 g.
5. $MgSO_4$	1.0 g.
6. Theobromin	0.05 g.
7. KH_2PO_4	2.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Adjustment of reaction not given.

Sterilization: Sterilize in autoclave, time not given.

Use: To study acid proofness of *B. tuberculosis*. On 19th day rods and diplococcoids were non acid proof. No growth using caffein instead of theobromin.

Reference: Wherry (1913 p. 151).

515. Bokorny's Picric Acid Solution

Constituents:

1. Water	1000.0 cc.
2. $Ca(NO_3)_2$ (0.02%)	0.2 g.
3. KH_2PO_4 (0.02%)	0.2 g.
4. $MgSO_4$ (0.02%)	0.2 g.
5. Picric acid (0.5%)	5.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of fungi, algae, amöbae and infusoria. No growth with 0.5% or 0.1% picric acid.

Variants: Bokorny used 0.1, 0.05, or 0.01% picric acid instead of 0.5%.

Reference: Bokorny (1917 p. 346).

SUBGROUP I-C. SECTION 7

Liquid media or basal solutions, containing organic carbon only as commercial digests; additional material, if any, inorganic.

A_1. Not containing peptone.
  Robinson and Rettger's Opsine Solution 516
  Linde's Nährstoff-Heyden Solution .. 517
  Lichtenstein's Cenovis Solution 518
A_2. Containing peptone.
B_1. Containing no added salts. (Exclusive of indicators or dyes.)
  Dunham's Peptone Solution (Comm. A. P. H. A.) 519
  Levine's Crystal Violet Peptone Solution 520
  Signorelli's Indicator Peptone Solution 521
  Stearn's Gentian Violet Peptone Solution 522
  Rivas' Trypsinized Peptone Solution 523
B_2. Containing added salts.
C_1. Containing salts of monovalent cations only.
D_1. Only one salt added
E_1. Sodium chloride only salt added.
F_1. No indicator added.
  Dunham's Peptone Solution 524
  Huss' Peptone Solution 525

- Nencki's Peptone Solution.......... 526
- Brussoff's Peptone Solution.......... 527
- Redfield's Peptone Solution (Meyers). 528
- Berman and Rettger's Peptone Solution.......... 529
- Migula's Peptone Solution.......... 530
- Heap and Cadness' Basal Peptone Solution.......... 531

F_2. Indicator added.

- Browning, Gilmour and Mackie's Brilliant Green Peptone Solution.. 532
- Harvey's Telluric Acid Peptone Solution.......... 533
- Smith's Rosalic Acid Peptone Solution.......... 534
- Bronfenbrenner's China Blue Peptone Solution.......... 535

E_2. Potassium (or sodium) nitrate only salt added.

- Diedudonne's Nitrate Peptone Solution.......... 536
- Harvey's Nitrate Peptone Solution.. 537

E_3. A salt other than E_1 or E_2 added.

- Cohen and Clark's Phosphate Peptone Solution.......... 538
- Enlow's Basal Agar Peptone Solution.......... 539
- Beijerinck's Ammonium Sulphate Peptone Solution.......... 540
- Redfield's Peptone Solution (Tanner).......... 541
- Nicolle, Raphael and Debains' Peptone Solution.......... 542
- Levine's Boric Acid Peptone Solution.......... 543

D_2. More than one salt added.

E_1. Containing phosphates.

- DeBord's Phosphate Peptone Solution.......... 544
- Stutzer's Nitrate Peptone Solution.. 545
- Kendall, Day and Walker's Fat Free Peptone Solution.......... 546
- Kligler's Peptone Solution.......... 547
- Klecki's Basal Ammonium Phosphate Peptone Solution.......... 548

E_2. Not containing phosphates.

- Pergola's Nitrate Peptone Solution.. 549
- Klimmer's Nitrate Peptone Solution. 550
- Bleisch's Nitrate Peptone Solution.. 551
- Logie's Nitrite Peptone Solution.... 552

C_2. Containing salts of divalent cations.

D_1. Containing calcium.

- Gottheil's Peptone Solution.......... 553
- Guyenot's Peptone Solution.......... 554
- Will's Peptone Solution.......... 555
- Gersbach's Trypsinized Peptone Solution.......... 556

D_2. Not containing calcium.

E_1. Containing iron salts.

- Kappen's Peptone Solution.......... 557
- Percival's Ammonium Sulphate Peptone Solution.......... 558

E_2. Not containing iron salts.

- Fremlin's Ammonium Sulphate Peptone Solution.......... 559
- Mortensen's Peptone Solution.......... 560
- Kligler's Nitrate Peptone Solution.. 561
- Percival's Phosphate Peptone Solution.......... 562
- Capaldi and Proskauer's Peptone Solution.......... 563
- Ritter's Peptone Solution.......... 564
- Speakman's Basal Peptone Solution (McCoy et al.).......... 565

516. Robinson and Rettger's Opsine Solution

Constituents:

1. Water.......... 1000.0 cc.
2. Opsine (1.0%).......... 10.0 g.

Preparation:

(1) Dissolve 1.0% opsine in water over a free flame.
(2) Adjust the reaction to +0.6 to phenolphthalein.
(3) Boil two or three minutes.
(4) Filter and distribute in 20.0 cc. lots in 150.0 cc. Erlenmeyer flasks.

Sterilization: Sterilize at 12 to 14 pounds pressure for 15 minutes.

Use: Cultivation of *B. diphtheriae* and study of toxin production. Using 2% opsine M.L.D. = 0.7 cc.

Variants:

(a) Used 2.0% opsine.
(b) Used 1.0% or 2.0% opsine with 1.0% glucose, and 0.5% NaCl.
(c) Used 1.0% or 2.0% opsine with 0.5% NaCl.

Reference: Robinson and Rettger (1917 p. 363).

517. Linde's Nährstoff Heyden Solution

Constituents:

1. Water.......... 1000.0 cc.
2. Nährstoff Heyden.......... 5.0 g.

Preparation: (1) Dissolve 2 in 1.

Sterilization: Not given.

Use: Cultivation of *Cladothrix.* Author reported good growth for 2 or 3 days. Then growth stopped.
Reference: Linde (1913 p. 386).

518. Lichtenstein's Cenovis Solution

Constituents:
1. Water
2. Cenovis

Preparation:
(1) Add cenovis (a fine yellow yeast preparation) to water (amount not given). Mix well.
(2) Allow to stand at room temperature for 30 minutes and then steam for 2 hours.
(3) Neutralize with soda solution and sterilize pin like steamer for another hour.
(4) Filter. Filtrate is clear golden yellow fluid.

Sterilization: Not specified.
Use: General culture medium. Substitute for meat peptone.
Reference: Lichtenstein (1923 p. 390).

519. Dunham's Peptone Solution (Committee A. P. H. A.)

Constituents:

1. Water (tap)	1000.0 cc.
2. Peptone	10.0 g.

Preparation:
(1) Weigh a sauce pan and measure 1000.0 cc. of tap water into it.
(2) Dissolve 10.0 g. of peptone in (1) when hot.
(3) Make up the loss due to evaporation by adding water.
(4) Filter twice thru the same filter.
(5) Distribute into tubes.

Sterilization: Autoclave for 10 minutes at 120°C.
Use: General culture medium. Indol test.
Variants:
(a) Buhlert and Fickendey used 1.5% peptone solution to show peptone decomposition by bacteria from the soil.
(b) Molisch used a 0.05% manganese peptone solution for the cultivation of iron bacteria *Leptothrix ochraceae,* etc.
(c) Remy and Rösing used a 0.8% solution of Witte's peptone or a 1.0% Merck peptone solution to study decomposition of peptone by bacteria from the soil.
(d) Linde used a 0.1 or 0.5% peptone solution to cultivate Cladothrix. Good growth was obtained for 2 or 3 days.

References: Committee A. P. H. A. (1905 p. 110), Heinemann (1905 p. 25), Buhlert and Fickendey (1906 p. 400), Molisch (1910 p. 36), Remy and Rösing (1911 p. 39), Linde (1913 p. 386), Tanner (1919 p. 47).

520. Levine's Crystal Violet Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (1.0%)	10.0 g.
3. Crystal violet	

Preparation:
(1) Dissolve 2 in 1.
(2) Add varying amounts of crystal violet.

Sterilization: Not specified.
Use: Differentiation of *Bact. coli* and *Bact. aerogenes.* The author reported that 1–100,000 crystal violet prevented growth of *Bact. coli,* while *Bact. aerogenes* grew heavily. Decreasing the concentration of peptone to 0.5% increased markedly the inhibitory action of the dye.
Reference: Levine (1911 p. 22).

521. Signorelli's Indicator Peptone Solution

Constituents:

1. Peptone water	1000.0 cc.
2. Dahlia (1.0% soln.)	5.0 cc.

Preparation:
(1) Exact composition of peptone water not given.
(2) Adjust (1) to neutral.
(3) Tube in 10.0 cc. lots.
(4) Add 0.05 cc. of 1.0% dahlia solution to each tube.

Sterilization: Not specified.
Use: To show adsorption of dye by cholera vibrio. The author reported that the vibrios developed rapidly and fell to the bottom of the tube. They were highly colored with dahlia and the medium was decolorized. With the other dyes the vibrio developed rapidly but did not color so strongly. The media were not decolorized.

Variants: Signorelli prepared 1.0% solutions of erythrosin or sapronin and added 1.0 cc. of one of the solutions to each tube of culture medium.

Reference: Signorelli (1912 p. 472).

522. Stearn's Gentian Violet Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	20.0 g.
3. Gentian violet	1:200,000

Preparation:

(1) Dissolve 2 in 1.

(2) Add 1 to 200,000 parts gentian violet.

(3) pH from 6.8 to 6.5.

Sterilization: Not specified.

Use: To study behavior of the colon aerogenes group with dyes. Author reported that the more basic the medium the more of the basic dye was absorbed by bacterial protein.

Variants: The author added 2.0 g. lactose.

Reference: Stearn and Stearn (1923 p. 568).

523. Rivas' Trypsinized Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte)	10.0 g.
3. Trypsin	5.0 g.

Preparation:

(1) Dissolve 10.0 g. Witte's peptone with gentle heating in 200–300 cc. of water.

(2) Dissolve 5.0 g. trypsin in 10–20 cc. water by shaking and heating not to exceed 40°C.

(3) Add (2) to (1).

(4) Incubate (3) at 38–40°C. for 2 to 3 hours, stirring gently every 15 to 20 minutes.

(5) Neutralize the reaction after the 2 or 3 hours if necessary.

(6) Add water to make up to 1000.0 cc.

(7) Boil and filter.

(8) Distribute in tubes.

Sterilization: Sterilize in the steamer or autoclave.

Use: Study of indol production.

Variants: Norton and Sawyer adjusted the reaction to +1.0 to phenolphthalein.

References: Rivas (1912 p. 549), Roddy (1917 p. 42), Norton and Sawyer (1921 p. 473).

524. Dunham's Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl (0.5%)	5.0 g.
3. Peptone (1.0%)	10.0 g.

Preparation:

(1) Autoclave 1, 2 and 3 to two atmospheres.

(2) Filter thru paper.

Sterilization: Not specified.

Use: Detection of cholera vibrio. Author reported that when concentrated H_2SO_4 was poured down the side of a tube containing a culture of the cholera bacillus, a red ring developed where the solutions came together. A 5.0 or 10.0% peptone solution gave better and quicker results.

Variants:

(a) Dunham used 5.0 or 10.0% peptone.

(b) Smith used the medium to determine indol and phenol production.

(c) Sears used 2.0% peptone.

(d) Myers used 3.0% Witte, Difco or Fairchild peptone. Used to study production of H_2S.

(e) Bezançon specified the use of Chapoteaut peptone.

(f) Percival used 2.0% peptone. Used to test for indol production.

(g) Norton and Sawyer specified Armours peptone.

(h) Harvey used from 1.0 to 2.0% peptone. At a pH = 8.0 to 9.0 the medium was used for the isolation of *V. cholerae*.

(i) Löhnis and Stitt specified the use of Witte's peptone.

(j) Wherry dialyzed the peptone for 2 days before using in the solution.

(k) Bristol used a 25% Difco peptone solution with 0.5% NaCl to study the metabolism of *B. botulinus*.

(l) Almy and James used a 3.0% peptone and 0.5% NaCl solution to study the volatile sulphur compounds produced by *Salmonella aertrycke* and *Proteus vulgaris*. They reported that when cysteine was added to this medium it was completely decomposed.

References: Dunham (1887 p. 338), Smith (1902 p. 94), Frost (1903 p. 64), Smith (1905 p. 195), Wherry (1905 p. 439), Abel

(1912 p. 23), Löhnis (1913, p. 44), Sears (1916 p. 110), Roddy (1917 p. 42), Besson (1920 p. 29), Myers (1920 p. 242), Bezançon (1920 p. 111), Percival (1920 p. 113), Buchan (1910 p. 108), Norton and Sawyer (1921 p. 473), Harvey (1921–22 p. 101), Giltner (1921 p. 42), Abbott (1921 p. 140), Dopter and Sacquépée (1921 p. 119), Pitfield (1922 p. 118), Stitt (1923 p. 35), Park, Williams and Krumwiede (1924 p. 120), Cunningham (1920 p. 18), Bristol (1925 p. 467), Almy and James (1926 p. 323).

525. Huss' Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: To study indol production by aroma producing bacteria. *Bacillus esterificans*, Maassen, and *Pseudomonas Trifolii*. Reaction for indol was negative when using H_2SO_4 and several drops of 1.0% KNO_2 as a test for indol.

Variants: Brussoff used this solution to cultivate iron bacteria from sludge, *Ferribacterium duplex*. A yellow membrane formed after 3 days.

Reference: Huss (1907 p. 61), Brussoff (1918 p. 195).

526. Nencki's Peptone Solution

Constituents:

1. Water	900.0 cc.
2. Peptone	100.0 g.
3. NaCl	20.0 g.

Preparation:

(1) Dissolve 100.0 g. peptone in 900.0 cc. water.

(2) Add 20.0 g. NaCl to (1).

(3) Filter.

(4) Distribute into test tubes.

Sterilization: Sterilize in the autoclave.

Use: To study cause of cattle plague. When the medium was inoculated with material containing the causative organism of cattle plague, round cells 1 to 3μ developed after 2 days.

Reference: Nencki (1898 p. 530).

527. Brussoff's Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Witte)	10.0 g.
3. Iron peptone (Merck)	1.0 g.
4. NaCl	10.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1 by heating in streaming steam for 30 minutes.

(2) Filter.

(3) Adjustment of reaction not specified.

Sterilization: Sterilize in the autoclave under 1.5 atmospheres pressure for 10 minutes.

Use: Cultivation of iron bacteria (sludge forms), *Ferribacterium duplex*.

Reference: Brussoff (1918 p. 196).

528. Redfield's Peptone Solution (Myers)

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte or Difco)	300.0 g.
3. NaCl	75.0 g.

Preparation:

(1) Boil 2 in 700.0 cc. tap water and 75.0 g. NaCl until as much of peptone as possible has gone into solution. (Redfield used KCl.)

(2) Cool rapidly and make up to 1 liter.

(3) Heat to boiling in a flask, plug and cool rapidly.

(4) Add 0.5% NaCl (to clear).

(5) Filter cold thru paper.

(6) Distribute in 150.0 cc. Erlenmeyer flasks in 10.0 cc. lots.

Sterilization: Sterilize at 15 pounds for 15 minutes.

Use: Detection of H_2S by bacteria from animal feces. Used lead acetate paper to detect H_2S production.

Reference: Myers (1920 p. 232–235).

529. Berman and Rettger's Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte)	5.0 g.
3. NaCl	5.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Tube in 10.0 cc. lots.

Sterilization: Not specified.

Use: To study bacterial nutrition and metabolism.

Variants:

(a) Authors used 1.0% of Witte's peptone. Other peptones were also used. Digestive Ferments Co., Park Davis, Armour, Eimer and Amend and Arlington Chemical Co.'s "aminoids" were used.

(b) Authors used 0.5% or 1.0% of one of the above mentioned peptones and added 0.25 g. Liebig's beef extract.

Reference: Berman and Rettger (1918 pp. 371–377, 392).

530. Migula's Peptone Solution

Constituents:

1. Distilled water	600.0 cc.
2. NaCl	0.5 g.
3. Peptone	5.0 to 15.0 g.

Preparation:

(1) Add 0.5 g. of NaCl to 600.0 cc. of distilled water and boil for 30 minutes.

(2) Add from 5.0 to 15.0 g. of peptone to hot (1).

(3) Filter.

(4) Tube.

Sterilization: Steam for 15 minutes.

Use: Chiefly for the detection of typhoid and cholera bacilli.

Reference: Migula (1901 p. 19).

531. Heap and Cadness' Basal Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte) (3.0%)	30.0 g.
3. NaCl (0.25%)	2.5 g.

Preparation:

(1) Dissolve 2 and 3 in 1 by steaming.

(2) Filter.

(3) Adjust to pH = 7.6.

Sterilization: Autoclave at 115°C. for 20 minutes.

Use: To study hydrogen sulphide production by *B. aertryke*.

Added nutrients: The authors added one of the following combinations:

(a) 2.0% glucose (sterilized by steaming as a 50% solution).

(b) 0.42% Na_2HPO_4.

(c) 0.42% Na_2HPO_4 + 2.0% glucose.

(d) 0.05% glucose.

(e) 0.05% glucose + 0.42% Na_2HPO_4.

(f) 0.025% glucose.

(g) 0.025% glucose + 0.42% Na_2HPO_4.

(h) 0.01, 0.25, 0.05 or 2.0% maltose.

(i) 0.05, 0.1 or 2.0% xylose.

(j) 2.0% sucrose.

Variants: The authors used the basic medium without any additions.

Reference: Heap and Cadness (1924–25 p. 80–90).

532. Browning, Gilmore and Machie's Brilliant Green Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Witte or Rostock)	20.0 g.
3. NaCl	5.0 g.
4. Brilliant green (Bayer's brilliant green extra cryst.)	

Preparation:

(1) Add 2 and 3 to 1.

(2) Steam in a Koch sterilizer for ¾ of an hour.

(3) Filter thru paper.

(4) Distribute in 5.0 cc. lots.

(5) Prepare a 1.0% stock solution of brilliant green (Bayer's brilliant green extra cryst), in distilled water.

(6) Just before use of medium a 1:10,000 dilution is prepared by adding 0.1 cc. of (5) to 9.9 distilled water.

Sterilization: Sterilize at 120°C. for 15 minutes (medium reacts faintly alkaline to litmus).

Use: Enrichment of Bacillus typhosus.

Variants: Harvey used 10.0 to 20.0 g. peptone and added 25.0 cc. of a 1–10,000 brilliant green solution.

References: Browning, Gilmour and Mackie (1913–14 p. 338), Harvey (1921–22 p. 91).

533. Harvey's Telluric Acid Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl	5.0 g.
3. Peptone	10.0 to 20.0 g.
4. Brilliant green (1–10,000 soln.)	
5. Telluric acid (1–1000 soln.)	

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Reaction of pH = 7.2.

(3) Tube in 10.0 cc. quantities.

(4) Add 0.1, 0.2, 0.35, 0.5 or 0.7 cc. of a

freshly prepared 1:10,000 brilliant green solution to each tube.

(5) Add 0.2 cc. of a 1:1000 telluric acid solution to each tube.

Sterilization: Not specified.

Use: Enrichment medium. The inositol fermenters were suppressed.

Reference: Harvey (1921–22 p. 91).

534. Smith's Rosolic Acid Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl	5.0 g.
3. Peptone	10.0 g.
4. Rosolic acid (0.5% soln. in 80.0% alcohol)	2.0 cc.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Filter.
(3) Prepare a 0.5% rosolic acid solution in 80.0% alcohol.
(4) Add 2.0 cc. of (3) to (2).
(5) Tube.

Sterilization: Sterilize the fractional method.

Use: To detect acid and alkali production. Medium is pale pink. Colorless with acid and red with alkaline reaction. Enrichment of colon typhoid group.

Variants:

(a) Harvey used 10.0 to 20.0 g. peptone.
(b) Bronfenbrenner added 0.05 to 62.5 cc. of a 2.0% solution of Grübler rosolic acid in 50.0% alcohol to the peptone solution. He recommended the use of 0.25% stock rosolic acid solution for enrichment of the intestinal bacteria.

References: Smith (1902 p. 94), Ball (1919 p. 83), Bronfenbrenner (1920 p. 84), Harvey (1921–22 p. 88).

535. Bronfenbrenner's China Blue Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl (c.p.)	5.0 g.
3. Peptone (Difco)	10.0 g.
4. China blue	

Preparation:

(1) Prepare a stock solution by dissolving 1.0 g. of Grübler's china blue in 100.0 cc. of 50.0% alcohol.
(2) Dissolve 2 and 3 in 1.
(3) Add 0.25% of (1) to (2).

Sterilization: Not specified.

Use: Enrichment of colon typhoid group. Using a mixture of dyes (china blue and rosalic acid). The author reported that the rosolic acid was the dye that inhibited the gram positive rods.

Variants: The author prepared a stock china blue and rosolic acid solution by dissolving 10.0 g. Grübler's china blue and 2.0 g. Grübler's rosolic acid in 100.0 cc. of 50.0% alcohol. This mixture of dyes (0.25%) was added to the medium instead of just china blue solution alone.

Reference: Bronfenbrenner (1920 p. 184).

536. Diedudonne's Nitrate Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. KNO_3 (0.01%)	0.1 g.
3. Peptone (1.0%)	10.0 g.

Preparation:

(1) Prepare a 1.0% slightly alkaline peptone solution.
(2) Add 0.01% KNO_3 to (1).
(3) Distribute in 10.0 cc. lots.

Sterilization: Method not given.

Use: Study of nitrate reduction by colon typhoid group.

Variants:

(a) Author used 0.001% or 0.0001% KNO_3.
(b) Grimbert specified the use of Colas peptone and used 1.0 g. KNO_3.
(c) Maassen used 5.0% peptone and 0.5% KNO_3.
(d) Maassen used 5.0% peptone, 0.5% KNO_3 and added 1.0% glycerol.
(e) Heinemann used 1.0 g. peptone and added 5.0 cc. of a 2.0% KNO_3 solution instead of 0.1 g. KNO_3.
(f) Committee A. P. H. A. and Harvey used 1.0 g. peptone and 2.0 g. KNO_3.
(g) Killer used 10.0 g. KNO_3.
(h) Rogers, Clark and Davis, Tanner, Ball, Giltner, and Park, Williams and Krumwiede used 1.0 g. peptone and 0.2 g. KNO_3.
(i) Committee S. A. B. used 2.0 to 5.0 g. peptone and 2.0 to 5.0 g. KNO_3.
(j) Tanner used 1.0 g. Witte's peptone and 2.0 g. KNO_3.
(k) Conn and Breed used 0.2 or 2.0 g. nitrate.

(l) Conn and Breed used 0.2 or 2.0 g. nitrate and added 10.0 g. glucose.
(m) Conn and Breed used 0.2 g. KNO_3 with 2.0 or 5.0 g. peptone.
(n) Conn and Breed used 0.2 g. KNO_3, 2.0 or 5.0 g. peptone and added 10.0 g. glucose.

References: Diedudonne (1894–95 p. 510), Grimbert (1898 p. 385), Maassen (1902 p. 28), Heinemann (1905 p. 131), Committee A. P. H. A. (1905 p. 109), Killer (1913 p. 521), Rogers, Clark and Davis (1914 p. 415), Committee S. A. B. (1918 p. 116), Tanner (1919 p. 90), Conn and Breed (1919 pp. 273–275), Tanner (1919 p. 45), Ball (1919 p. 76), Giltner (1921 p. 42), Harvey (1921–22 pp. 102, 108), Park, Williams and Krumwiede (1924 p. 124).

537. Harvey's Nitrate Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $NaNO_3$	1.0 g.
3. Peptone	10.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: Study of nitrate reduction.

Reference: Harvey (1921–22 p. 107).

538. Cohen and Clark's Phosphate Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Difco)	10.0 g.
3. K_2HPO_4	5.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Adjust to any desired pH value by the addition of HCl or NaOH.

Sterilization: Not specified.

Use: To study effect of reaction (pH) on growth of the colon-aerogenes group, and to study ammonification.

Variants: Cunningham studied ammonification using the following soil and manure forms, *Bac. mycoides*, *Bact. fluorescens*, *Bact. vulgare*, *Bact. coli*, *Sarcina lutea*, *Bac. putrificus*. He specified the use of tap water and used 0.05% K_2HPO_4 instead of 0.5%.

Reference: Cohen and Clark (1919 p. 410).

539. Enlow's Basal Agar Peptone Solution

Constituents:

1. Water (tap or distilled)	2000.0 cc.
2. Peptone	10.0 g.
3. K_2HPO_4	17.0 g.
4. Agar	2.0 g.
5. Indicator (Brom thymol blue)	

Preparation:

(1) Dissolve 2, 3 and 4 in 1800.0 cc. water in the Arnold sterilizer by heating for 40 minutes or autoclave for 10 minutes or autoclave for 10 minutes at 15 pounds pressure.
(2) Filter through paper while hot.
(3) Dilute to 2000.0 cc. by the addition of hot distilled water.
(4) Adjust the hydrogen ion concentration as desired.
(5) Add 0.5% of one of the added nutrients.
(6) Add any desired indicator. (The author added 3 drops of Brom thymol blue to each 500.0 cc. of medium. The indicator solution was prepared by grinding 0.1 gram Brom thymol Blue and 3.2 cc. of N/20 NaOH in an agate mortar. When solution of the dye was complete, add 3.0 cc. of distilled water.
(7) Tube.

Sterilization: Sterilize for 30 minutes in the Arnold sterilizer on each of 3 successive days.

Use: Used as a basic sugar free medium.

Variants: The author used 14.5 g. Na_2HPO_4 and 1.4 g. KH_2PO_4 instead of 17.0 g. K_2HPO_4. The combination of Na_2HPO_4 and KH_2PO_4 gave a medium with a pH of between 7.2 and 7.4.

Added nutrients: The author added 0.5% of any desired sugar, alcohol, glucose, etc.

Reference: Enlow (1923, p. 2130).

540. Beijerinck's Ammonium Sulphate Peptone Solution

Constituents:

1. Water (ditch)	1000.0 cc.
2. Peptone	5.0 g.
3. $(NH_4)_2SO_4$	1.0 g.

Preparation:

(1) Dissolve 2 and 3 in ditch water ("Grabenwasser").
(2) Make alkaline with Na_2CO_3.

Sterilization: Not specified.

Use: To study reduction of sulphates by *Spirillum tenue*, generally the more organic material that is present the greater the reduction.

Variants: Cathelineau studied pigment and fluorescent production by *Bacillus varidis* (Lesage) and used Beijerinck's solution with one of the following:

glucose	5.0 g.
sodium phosphate	5.0 g.
sodium succinate	5.0 g.

Reference: Beijerinck (1895 p. 107), Cathelineau (1896 p. 235).

541. Redfield's Peptone Solution (Tanner)

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone	300.0 g.
3. KCl	74.0 g.

Preparation:

(1) Add 2 and 3 to hot 700.0 cc. distilled water.
(2) Stir until solution is complete.
(3) Cool.
(4) Allow to stand in the ice box over night.
(5) Filter.
(6) Make up to one liter.
(7) Tube.

Sterilization: Not specified.

Use: General culture medium.

Reference: Tanner (1919 p. 59).

542. Nicolle, Raphael and Debaens Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Defresne)	25.0 g.
3. Soda (normal)	20.0 cc.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Tube in 4.0 cc. lots.

Sterilization: Sterilize at 110°C. for 15 minutes.

Use: Cultivation of Eberth's bacillus and paratyphoid bacilli. Indol was not produced in this medium.

Reference: Nicolle, Raphael and Debains (1917 p. 380).

543. Levine's Boric Acid Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (1.0%)	10.0 g.
3. Boric acid (H_3BO_3 0.63%)	6.3 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: To determine the effect of boric acid on the growth of *Bact. coli* and *Bact. aerogenes*. The author reported that *Bact. aerogenes* died off in this medium but that *Bact. coli* multiplied slowly.

Reference: Levine (1911 p. 22).

544. DeBord's Phosphate Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Difco)	32.0 g.
3. KH_2PO_4 (M/2 solution)	16.0 cc.
4. Na_2HPO_4 (M/2 solution)	144.0 cc.

Preparation:

(1) Dissolve peptone in 900.0 cc. cold distilled water.
(2) Add 16.0 cc. half molar KH_2PO_4 and 144.0 cc. of half molar Na_2HPO_4 solutions.
(3) Dilute to 1500.0 cc.
(4) Heat for 15 minutes at 15.0 pounds pressure.
(5) Filter and make up loss in weight.
(6) Add 100.0 cc. distilled water.

Sterilization: Sterilize to 15 pounds pressure for 30 minutes.

Use: To study nitrogen metabolism of *B. coli*, *Ps. pyocyanea*, *C. sporogenes* and *P. subtilis*.

Variants: The author added 100.0 cc. of a sterile glucose solution prepared by dissolving 16.0 g. glucose in distilled water instead of the 100.0 cc. distilled water.

Reference: DeBord (1923 pp. 16, 17).

546. Kendall, Day and Walker's Fat Free Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Fairchilds)	15.0 g.
3. Na_2HPO_4	3.0 g.
4. NaCl	5.0 g.

Preparation:

(1) Extract 15.0 g. of 2 for two weeks with ether, for two weeks with alcohol, two weeks with acetone and 10 days with petroleum ether, respectively, in a Soxhlet extractor, the successive extractions occurring at intervals of

about 12 minutes. These extractions to be continued for 6 hours a day and 6 days per week.
(2) Dissolve residue from the extraction in 1.
(3) Dissolve 3 and 4 in (2).
(4) Distribute in 100.0 cc. lots.
(5) Adjustment of reaction not given.

Sterilization: Autoclave (time not given).

Use: To study metabolism of tubercle bacilli. Authors used alizarin, neutral red, and phenolphthalein to study changes in reaction. Ziehl-Neelsen stain for staining.

Variants: Harvey used 30.0 g. of peptone (did not specify Fairchilds) and added 0.1 cc. of 1.0% neutral lead acetate to each 10.0 cc. quantity of medium.

References: Kendall, Day and Walker (1914 p. 424), Harvey (1921–22 p. 107).

547. Kligler's Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Witte's)	10.0 g.
3. K_2HPO_4	0.29 g.
4. NaCl	5.0 g.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Adjustment of reaction not given.
(3) Filter thru filter paper.
(4) Tube in 10.0 cc. lots.

Sterilization: Autoclave (time not given).

Use: To study indol production.

Reference: Kligler (1914 p. 82).

548. Klecki's Basal Ammonium Phosphate Peptone Solution

Constituents:

1. Water	5000.0 cc.
2. Peptone	10.0 g.
3. Potassium phosphate	0.29 g.
4. Ammonium phosphate	0.5275 g.
5. $MgSO_4$	0.275 g.
6. $(NH_4)_2SO_4$	0.115 g.

Preparation:
(1) Dissolve 2, 3, 4, 5, 6 and 100.0 g. of one of the added nutrients in 1.
(2) Distribute in Pasteur fermentation tubes.

Sterilization: Method not given.

Use: Cultivation of *Bacillus saccharobutyricus*. Organism did not grow using calcium lactate as a carbon source.

Added nutrients: The author added 100.0 g. of lactose or 100.0 g. of calcium lactate.

Reference: Klecki (1896 p. 255).

549. Pergola's Nitrate Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	10.0 g.
4. Na_2CO_3	0.2 g.
5. KNO_3	0.1 g.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Filter.
(3) Distribute in 15 to 20.0 cc. lots.

Sterilization: Method not given.

Use: Enrichment of cholera vibrio.

Variants:
(a) Wölfel used 2.0% peptone, 0.5% NaCl, 0.0075% KNO_3 and 0.2 g. Na_2CO_3 in 100.0 cc. distilled water to demonstrate the cholera red reaction.
(b) Ball used 10.0% peptone, 0.1% KNO_3 and 0.1% Na_2CO_3.

References: Pergola (1911 p. 85), Wölfel (1912 p. 416), Ball (1919 p. 83).

550. Klimmer's Nitrate Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Witte)	100.0 g.
3. NaCl	100.0 g.
4. KNO_3	1.0 g.
5. Na_2CO_3 (crystalline)	2.0 g.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1000.0 cc. distilled water.
(2) Tube or flask.

Sterilization: Method not given.

Use: Enrichment of cholera vibrio. Add 9 parts of water under investigation to 1 part of medium.

Reference: Klimmer (1923 p. 221).

551. Bleisch's Nitrate Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Witte)	20.0 g.
3. NaCl (c.p.)	5.0 g.
4. KNO_3 (c.p.)	0.8 g.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Boil.
(3) Filter.

Sterilization: Method not given.

Use: Study of indol production by cholera bacteria.

Variants:

(a) Frost and Johnson used 1.0 g. Merck's peptone, 0.2 g. KNO_3 and 0.5 g. NaCl.

(b) Wherry used 10.0 g. of Witte's peptone and 0.01% KNO_3.

(c) Wherry used 10.0 g. of Witte's peptone that was dialysed for two days and 0.01% KNO_3.

(d) Logie used 10.0 g. peptone and 0.00283 g. KNO_3.

(e) Cunningham used 10.0 g. peptone, 5.0 g. NaCl and 0.5 g. nitrite free KNO_3.

Reference: Bleisch (1893 p. 112), Frost (1903 p. 66), Wherry (1905 p. 439), Logie (1919 p. 147), Johnson (1912 p. 219), Cunningham (1924 p. 18).

552. Logie's Nitrite Peptone Solution

Constituents:

1. Water	1000.0	cc.
2. Peptone	10.0	g.
3. NaCl	5.0	g.
4. $NaNO_2$	0.0023	g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: To study the formation and destruction of nitrite by the dysentery bacilli.

Reference: Logie (1910 p. 147).

553. Gottheil's Peptone Solution

Constituents:

1. Distilled water	1000.0	cc.
2. Potassium phosphate	1.0	g.
3. $CaCl_2$	0.1	g.
4. $MgSO_4$	0.3	g.
5. NaCl	0.1	g.
6. Iron	trace	
7. Peptone	10.0	g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: General culture medium for organisms found in the soil, on roots and rhizomes.

Reference: Gottheil (1901 p. 432).

554. Guyenot's Peptone Solution

Constituents:

1. Redistilled water	1000.0	cc.
2. Na_2SO_4	0.16	g.
3. $CaHPO_4$	0.32	g.
4. $MgCl_2$	0.56	g.
5. KH_2PO_4	4.16	g.
6. Peptone (Chapoteaut)	20.0	g.

Preparation:

(1) Add 2, 3, 4 and 5 to 1.

(2) Add sufficient acetic acid to keep the materials in solution.

(3) Dissolve 6 in (2).

Sterilization: Not specified.

Use: Cultivation of *Drosophila ampelophila.*

Reference: Guyenot (1914 p. 548).

555. Will's Peptone Solution

Constituents:

1. Distilled water	1000.0	cc.
2. $CaHPO_4$	0.5	g.
3. KH_2PO_4	4.55	g.
4. $MgSO_4$	2.1	g.
5. Witte peptone	20.0	g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of non-spore forming yeast.

Variants:

(a) Will used 4.55 g. K_2HPO_4 instead of KH_2PO_4 to cultivate mycoderma.

(b) Nakozawa added 5.0% sucrose.

Reference: Will (1908 p. 387), (1901 p. 3), Nakazawa (1909 p. 530).

556. Gersbach's Trypsinized Peptone Solution

Constituents:

1. Water	1000.0	cc.
2. Peptone (Witte)	10.0	g.
3. NaCl	0.5	g.
4. Calcium phosphate	0.2	g.
5. $MgSO_4$	0.02	g.

Preparation:

(1) Prepare a 1.0% solution of Witte's peptone and add 0.5% NaCl to the liter.

(2) Boil one hour and cool to 40°C.

(3) Neutralize and make slightly alkaline with soda.

(4) Place in flask with a well fitting glass stopper.

(5) Add 0.2 g. of Grübler's trypsin, 0.2 g. calcium phosphate 0.02 f. $MgSO_4$, 10.0 cc. chloroform and 10.0 cc. of toluol.

(6) Shake thoroly, removing the stopper occasionally.
(7) Place a paper around the flask and place in the incubator at 37°C. for 24 hours.
(8) Shake thoroly.
(9) Filter thru a sterile moistened filter paper.

Sterilization: Sterilize for 45 minutes (method not given).

Use: Study of indol production. Culture medium used in water analysis.

Reference: Gersbach (1922 p. 146).

557. Kappen's Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (0.5%)	5.0 g.
3. K_2HPO_4	usual amounts
4. NaCl	
5. $MgSO_4$	
6. $FeSO_4$	

Preparation: (1) Prepare a 0.5% solution of peptone in water containing the usual amounts of K_2HPO_4, NaCl, $MgSO_4$, and $FeSO_4$ (amounts not given).

Sterilization: Method not given.

Use: To study decomposition of peptone by cyanamide decomposing bacteria.

Reference: Kappen (1909 p. 395).

558. Percival's Ammonium Sulphate Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2SO_4$	2.0 g.
3. K_2HPO_4	1.0 g.
4. NaCl	2.0 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.4 g.
7. $MgCO_3$	10.0 g.
8. Peptone (0.01%)	0.1 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Distribute in 50.0 cc. into conical flasks.
(3) Add 0.5 g. of basic $MgCO_3$ to each flask.
(4) Add 0.01% peptone to each flask.

Sterilization: Not specified.

Use: To study nitrification.

Variants: The author used 0.03, 0.3, 1.0 or 3.0% peptone instead of 0.01%.

Reference: Percival (1920 p. 144).

559. Fremlin's Ammonium Sulphate Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2SO_4$	1.0 g.
3. Potassium phosphate	1.0 g.
4. $MgCO_3$	10.0 g.
5. Peptone	0.1 to 0.5 g.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Dissolve 4 in the remainder of 1.
(3) Mix sterile (1) and (2) under aseptic conditions and add from 0.1 to 0.5 g. peptone.

Sterilization: Method not given.

Use: To study nitrogen oxidation by nitroso bacteria.

Reference: Fremlin (1903 p. 368).

560. Mortensen's Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte (1.0%))	10.0 g.
3. Cobalt salts	

Preparation:

(1) Dissolve 2 in 1.
(2) Add cobalt salts in varying amounts from 4 to 1/32%.

Sterilization: Not specified.

Use: To study the effect of cobalt salts on *Aspergillus niger*. The author reported that the toxic properties of cobalt were different in different media. A 0.5% cobalt chloride solution in a liquid medium was as toxic as 1.0% cobalt chloride in a gelatin medium.

Reference: Mortensen (1909 p. 523).

561. Kligler's Nitrate Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone	10.0 g.
3. K_2HPO_4	0.2 g.
4. $MgSO_4$	0.1 g.
5. NaCl	0.2 g.
6. KNO_3	0.2 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: To study denitrification by cocci. Author reported that cocci reduced nitrates to nitrites only. Ammonia was formed but as a decomposition product of the peptone.

Variants: The KNO_3 was omitted.
Reference: Kligler (1913 p. 790).

562. Percival's Phosphate Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4	2.0 g.
3. Magnesium phosphate	0.1 g.
4. Peptone	10.0 g.

Preparation:
(1) Dissolve 2 and 3 in 1.
(2) Add 4 to (1).
(3) Heat to boiling.
(4) Filter.
(5) Adjustment of reaction not specified.
(6) Tube in 10 to 15.0 cc. quantities.
(7) After sterilization inoculate with contents of a nodule of a pea, runner bean, red clover, and broad bean by means of a sterile knife.

Sterilization: Sterilize on 3 successive days (time not specified).

Use: Cultivation of bacteria from the nodules of legumes.

Reference: Percival (1920 p. 204).

563. Capaldi and Proskauer's Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl (0.02%)	0.2 g.
3. $MgSO_4$ (0.01%)	0.1 g.
4. K_2HPO_4 (0.2%)	2.0 g.
5. Peptone (Witte 0.5%)	5.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of colon-typhoid group.

Variants:
(a) The author used 2.0% peptone for 0.5% and used either 0.5 or 2.0% of one of the following instead of Witte's peptone.
Peptone (e Carne König)
Peptone (Aschman)
Peptone (Chapoteaut)
Peptone, drüsen (Kühne)
Hemialbumose (Grübler)
Somatose
Peptone (Glutin (Paal))
(b) Percival used the following solution to determine ammonia production by *B. mycoides:*

1. Water	1000.0 cc.
2. K_2HPO_4	5.0 g.
3. $MgSO_4$	2.5 g.
4. NaCl	2.5 g.
5. Peptone (0.1%)	1.0 g.

References: Capaldi and Proskauer (1896 p. 460), Percival (1920 p. 112).

564. Ritter's Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Witte)	10.0 g.
3. $MgSO_4$	1.0 g.
4. K_2HPO_4	1.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of *Bacillus prodigiosus* yeast and iron bacteria.

Variants:
(a) The author added 10.0 to 20.0% of glucose and specified that other sugars might be added instead of glucose if desired.
(b) Zikes cultivated *Cladothrix dichotoma* and *Cladothrix natans* in the following solution.

1. Water	1000.0 cc.
2. $MgSO_4$	1.0 g.
3. K_2HPO_4	0.25 g.
4. Peptone	1.25 g.

He substituted a nearly ash and iron free peptone or a "Manganpeptone" for ordinary peptone and reported slightly poorer growth using the ash and iron free peptone.
(c) Bokorny cultivated yeast in the following solutions:

1. Water	1000.0 cc.
2. Peptone	50.0 g.
3. KH_2PO_4	1.0 g.
4. $MgSO_4$	1.0 g.

or

1. Water	1000.0 cc.
2. KH_2PO_4	4.0 g.
3. $MgSO_4$	1.0 g.
4. Peptone	25.0 g.

References: Ritter (1900 p. 207), Zikes (1915 p. 542), Bokorny (1917 p. 370), (1920 p. 23).

565. Speakman's Basal Peptone Solution (McCoy et al.)

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	0.5 g.
3. KH_2PO_4	0.5 g.

4. $MgSO_4$	0.2 g.
5. NaCl	0.01 g.
6. $MnSO_4$	0.01 g.
7. Peptone	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Dissolve 3% of one of the added nutrients.

(3) Adjust the reaction to pH = 7.0.

Sterilization: Not specified.

Use: Cultivation of acetone butyl alcohol organisms.

Added nutrients: The authors added 3% of any desired carbohydrate.

Reference: McCoy, Fred, Peterson and Hastings (1926 p. 460).

SUBGROUP I-C. SECTION 8

Incomplete or basal solutions containing peptone as the sole organic nitrogen and carbon source; employed with the addition of a variety of materials.

A_1. No salts added.
B_1. Indicators employed.
  Morishima's Basal Phenol Red China Blue Peptone Solution 566
  Robinson's Basal Azolitmin Peptone Solution 567
B_2. Indicator not employed.
  Capaldi and Proskauer's Basal Peptone Solution 568
A_2. Salts added.
B_1. Only one salt added.
C_1. Salts of only monovalent cations employed.
D_1. Sodium salt added.
E_1. Indicator employed.
  Barsiekow's Basal Salt Peptone Solution 569
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566. Morishima's Basal Phenol Red China Blue Peptone Solution

Constituents:

1. Peptone solution	1000.0 cc.
2. Phenol red (0.02% soln.)	50.0 cc.
3. China blue decolorized (1.0% soln.)	12.0 cc.

Preparation:

(1) Method of preparation of 1 not given. Any fluid medium used to determine fermentation may be used.

(2) Prepare a 1.0% solution of China blue in distilled water, and keeping the water hot on a water bath, normal NaOH solution is slowly added until the blue color disappears and the solution becomes brown. (About 3.5 cc. N/1 NaOH is required per 100.0 cc. of China blue.) Keep in rubber stoppered flask.

(3) Prepare a 0.02% solution of phenol red.

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* See C_3.

(4) Adjust (1) to desired pH.
(5) Add 5.0 cc. of (3) and 1.2 cc. of (2) to (4) for every 100.0 cc. of (4).
(6) Add one of the added nutrients.

Sterilization: Autoclave at 10 pounds pressure for 10 minutes.

Use: To determine fermentation of sugars. Pink color indicates alkali formation. Acid indicated by first a bright green and then changing to deep blue.

Added nutrients: Author suggested the use of any carbohydrate or fermentable material. The carbohydrate solution may be sterilized separately and added aseptically to sterile (5), or the sugar can be added to sterilized (5) and the mixture be heated a second time in the autoclave at 10 pounds for 10 minutes.

Reference: Morishima (1920 p. 43).

567. Robinson's Basal Azolitmin Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte)	10.0 g.
3. Azolitmin	0.5 g.

Preparation:
(1) Prepare 500.0 cc. of a 2.0% solution of Witte's peptone.
(2) Adjust to a reaction of +0.6 to phenolphthalein.
(3) Prepare 500.0 cc. of a solution containing 2.0% of an added nutrient (sugar).
(4) Mix sterile (2) and (3) in equal amounts, and add 0.05% azolitmin.
(5) Tube in sterile tubes.

Sterilization: Sterilize (2) in the autoclave. Filter (3) thru Berkefeld candles into sterile flasks. Incubate 24 hours to insure sterility.

Use: To determine fermentation by typhoid and paratyphoid bacilli.

Added nutrients and variants:
(a) Robinson used 10.0 g. of any desired sugar.
(b) Harvey used 2.0 to 10.0 g. peptone, 50.0 cc. of litmus solution and 10.0 g. of any desired carbon source or fermentable material.
(c) Harvey used 20.0 g. peptone, 50.0 cc. of litmus solution and 10.0 g. of any carbon source or fermentable materials per 850.0 cc. of distilled water.

References: Robinson (1915 p. 407), Harvey (1921–22 pp. 101, 109).

568. Capaldi and Proskauer's Basal Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte) (0.5 or 2.0%)	5.0 or 20.0 g.

Preparation: (1) Dissolve 2 and 0.1% of one of the added nutrients in 1.

Sterilization: Not specified.

Use: To determine fermentation of sugars by the colon-typhoid group. Different investigators added various materials to the basic solution, or a modification of it, and used the media for a variety of purposes.

Added nutrients and variants:
(a) The authors added 0.1% of one of the following materials:
glucose
levulose
mannose
galactose
sorbose
lactose
maltose
melibiose
trehalose
raffinose
dextrin
inulin
mannitol
adonitol
sorbitol
isodulcitol
erythritol
dulcitol
They reported growth poor with a M.L.D. = 1.0+ cc.
(b) Matzuschita used 10.0 g. Koch's meat peptone per liter water as a basic solution and added one of the following materials or combinations:
NaCl 0.5, 0.7 to 10.5%.
NaCl 5.0 g. and glucose 0.5 to 50.0%.
NaCl 5.0 g., glucose 2.0% and from 0.2 to 15.0% Na_2CO_3. Add the Na_2CO_3 to the neutral medium.
NaCl 5.0 g., glucose 2.0%, glycerol 6.0%.
NaCl 5.0 g., glucose, 2.0% and from 0.1 to 0.4% HCl. Add the HCl to the neutral medium.
No additions to the basic solution. These media were used to cultivate *Clostridium butyricum*, *Bacillus oedematis maligni*, *Bacillus anthracis symptomatici*, *Bacillus sporogenes*, *Bacillus botulinus*.
(c) Revis did not specify the exact composition of the basic solution (pep-

tone water) and added one of the following materials:

mucic acid (sodium salt)	1.0%
saccharic acid (potassium salt)	1.0%
gluconic acid (potassium salt)	0.5%

The media were used to study the fermentation of acids by the colon group. The author reported that with 1.0% of the acid sodium salt of mucic acid no gas was produced with *B. coli* or *B. lactis aerogenes*, but slightly more acid was produced. With the normal sodium salt and in the presence of $Mg(OH)_2$ acid and gas were produced by *B. coli* and *B. lactis aerogenes.*

Same thing was true with saccharic acid. (Acid and gas produced when medium was alkaline.) Gluconic acid was attacked when present as the potassium salt or when alkalinity was increased to 1.0 cc. N/1 NaOH per 100.0 cc., or when the acid itself was employed.

(d) Davis and Ferry cultivated *Bact. diphtheriae* and studied toxin production using 20.0 g. of peptone in the basic solution and adding one of the following materials:

glycocoll	0.75 g.
leucine	30.0 g.
histidine dichloride	0.5 g.
glutaminic acid hydrochloride	2.5 g.
tyrosine	20.0 g.
cystine	0.5 g.
tryptophane	0.6 g.
sodium asparaginate	1.5 g.
creatine 0.2 g + creatinine 0.15 g.	
xanthin 0.05 g. + hypoxanthine 0.05 g.	
glucose amine hydrochloride	2.0 g.

(e) Holm and Sherman used 10.0 g. of peptone per liter in their basic solution and added one of the following salts in sufficient quantities to make the medium 0.2 molar for each salt.

NaCl
$NaNO_3$
$NaHPO_4$
NaI
Na_2SO_4
Na lactate
Na oxalate
Na acetate
Na citrate
Na fluoride
KCl
$CaCl_2$
$MgCl_2$
$FeCl_3$
NH_4Cl

In case of sodium lactate, NaOH is added to lactic acid until a pH = 7.0 is reached. In case of the phosphates, mono and di-sodium phosphates are mixed in the proper proportions to give a pH of approximately 7.0. NaCl is used also in 0.4M and $MgCl_2$ in 0.1M concentrations. The media were used to show the effect of salts on the growth of *B. coli.* The authors reported that generally the order of growth acceleration of *B. coli* by these salts was approximately the same as their order in the lyotropic series.

(f) Hotchkiss used 10.0 g. peptone per liter in the basic solution and added a number of salts to study the effect of various cations on a communis type of *B. coli.* He used the following salts and reported that the concentrations here indicated stimulated growth. NaCl, KCl, $CaCl_2$ and $MgCl_2$ may be added to the peptone before sterilization. Other salt solutions were made up and stored until sterile and added under aseptic conditions to the sterile peptone solution. pH for all solutions except NH_4Cl were from 6.6 to 7.0. NH_4Cl solution pH = 6.0 to 6.4.

NaCl	0.25 molar concentration in the media.
KCl	0.25 molar concentration in the media.
NH_4Cl	0.25 molar concentration in the media.
$CaCl_2$	0.05 molar concentration in the media.
$MgCl_2$	0.05 molar concentration in the media.
$SrCl_2$	0.025 molar concentration in the media.
$BaCl_2$	0.05 molar concentration in the media.
$TiCl_2$	0.0005 molar concentration in the media.
$NiCl_2$	0.0001 to 0.00005 molar concentration in the media.
$PbCl_2$	0.0005 to 0.00005 molar concentration in the media.
$SnCl_2$	0.00005 to 0.000005 molar concentration in the media.
$ZnCl_2$	0.00005 to 0.00001 molar concentration in the media.

$CeCl_2$ 0.00001 molar concentration in the media.

$HgCl_2$ 0.000001 molar concentration in the media.

References: Capaldi and Proskauer (1894 p. 467), Matzuschita (1902 p. 286), Revis (1912 p. 427), Davis and Ferry (1919 p. 235), Holm and Sherman (1921 p. 515), Hotchkiss (1923 pp. 142–161).

569. Barsiekow's Basal Salt Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Lackmus tincture	100.0 g.
3. Peptone (1.0%)	10.0 g.
4. NaCl (0.5%)	5.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 1.0% of one of the added nutrients in 1.

Sterilization: Not specified.

Use: Differentiation of colon typhoid group. Colon forms turned the medium red, typhoid and alkaligenes types blue.

Added nutrients and variants:

(a) The author added 1.0% glucose or 1.0% lactose.

(b) Harvey used a similar basic solution and prepared it as follows:

 (1) Dissolve 10.0 g. peptone, 5.0 g. NaCl in a liter of water by steaming.
 (2) Make faintly alkaline to litmus by the addition of sodium carbonate.
 (3) Filter.
 (4) Sterilize.
 (5) Place solid commercial litmus in a flask and pour 96% alcohol over it.
 (6) Close (5) with a rubber stopper.
 (7) Keep in an incubator at 30°C. for 2 days, shaking occasionally.
 (8) Pour off the alcohol and add fresh.
 (9) Continue the process until the alcohol extracts no more color.
 (10) Filter off the alcohol.
 (11) Dry the residue.
 (12) Dissolve (11) in distilled water to saturation.
 (13) Add sufficient 1–1000 sulphuric acid to produce the slightest color.
 (14) Dissolve 10.0 g. of any desired carbon source of fermentable material in 50.0 cc. of (13).
 (15) Add (14) to (4).
 (16) Filter into test tubes.
 (17) Sterilize for 2 days at 100°C. and allow the temperature to reach 120°C. on the third day and then extinguish the flame.

References: Barsiekow (1901 pp. 823–825), Harvey (1921–22 p. 108).

570. Cunningham's Basal Fuchsin Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.
4. Andrades indicator (1.0%)	10.0 cc.

Preparation:

(1) Dissolve 2 and 3 in 1 by autoclaving to 2 atmospheres pressure.
(2) Filter thru paper.
(3) Dissolve 0.5% of one of the added nutrients and 1.0% of Andrades indicator in (2).
(4) Tube with Dunham's fermentation tubes.
(5) Stand the tubes in a water bath and heat to boiling.
(6) Plug.

Sterilization: Sterilize in the autoclave at 22.5 pounds pressure. (Do not exceed 22.5 pounds pressure.)

Use: To observe fermentation.

Added nutrients: The author added 0.5% of any desired sugar.

Reference: Cunningham (1924 p. 18).

571. Harvey's Basal Neutral Red Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl	5.0 g.
3. Peptone	10.0 g.
4. 1.0% neutral red	2.5 cc.

Preparation:

(1) Dissolve 2 and 3 in 1 by steaming.
(2) Filter.
(3) Add freshly prepared 1.0% neutral red 2.5 cc.
(4) Prepare a 10.0% solution of one of the added nutrients.
(5) Add sufficient amount of sterile (4) to sterile (3) so that the concentration of the added nutrient is 1.0%.
(6) Distribute in test tubes containing fermentation tubes.

Sterilization: Sterilize (3) in the autoclave. Sterilize (4) at 105°C. for 10 minutes. Sterilize the tubes at 105°C. for 10 minutes or at 100° on each of 2 successive days for 20 minutes.

Use: To observe fermentation by bacteria.

Added nutrients: The author did not specify the sugars used. One per cent of any desired carbon source may be employed.

Reference: Harvey (1921–22 p. 108).

572. Abba's Basal Phenolphthalein Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	100.0 g.
3. NaCl	50.0 g.
4. Phenolphthalein (1.0% alcoholic soln.)	0.5 cc.

Preparation:

(1) Dissolve 2, 3 and 200.0 g. of one of the added nutrients in 1.

(2) Boil in the steamer for 30 minutes at 100°C.

(3) Filter.

(4) Distribute into 100.0 cc. lots into sterile containers.

(5) After sterilization add 0.5 cc. of phenolphthalein solution and Na_2CO_3 solution until the solution is permanently red (see use).

Sterilization: Not specified.

Use: Isolation of *Bacillus coli communis* from water. About 1 liter of the water under investigation is added to each flask of medium. *Bacillus coli communis* decolorized the solution after from 12 to 24 hours (using glucose). Using lactose *B. coli* caused decolorization after about 8 to 24 hours. Typhoid bacilli require a greater length of time.

Added nutrients: The author added 200.0 g. of lactose or glucose.

Reference: Abba (1896 p. 13), (1896 p. 224), (1895 #176).

573. Mendel's Basal Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Witte)	10.0 g.
3. NaCl	5.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add 1.0% of one of the added nutrients (see sterilization).

Sterilization: Sterilize for 30 minutes on each of 2 successive days. On the third day add 1.0% of one of the added nutrients, and sterilize for 15 minutes.

Use: To study decomposition of sugars. Other investigators used variants of the medium to cultivate sludge forms and to study proteolysis.

Added nutrients and variants:

(a) Mendel added 1.0% of one of the following:

glucose	lactose
sucrose	maltose

(b) Rettger, Berman and Sturges studied proteolysis used 5.0 g. NaCl and 2.5, 5.0 or 20.0 g. of Witte's peptone in the basic solution and adding the following materials individually or in combination:

glucose	10.0 g.
beef extract	5.0 g.
$(NH_4)_2SO_4$	2.5 g.

(c) Brussoff used 10.0 g. NaCl in the basic solution. He used the media to cultivate *Ferribacterium duplex*, and other iron bacteria from sludge. He added one of the following materials to the basic solution.

Iron ammonium citrate	0.5 g.
K_2CO_3	1.0 g.
K_2SO_4	1.0 g.
Potassium acetate	1.0 g.
Potassium citrate	1.0 g.

References: Mendel (1911 p. 297), Brussoff (1918 pp. 195, 208), Rettger, Burman and Sturges (1916 p. 20–22).

574. Nicolle, Raphael and Debains' Basal Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Defresne)	1.0 g.
3. Soda (normal)	20.0 cc.

Preparation:

(1) Dissolve 2, 3 and one of the added nutrients in 1.

(2) Distribute in test tubes containing fermentation tubes.

Sterilization: Sterilize at 110° for 15 minutes.

Use: To study the fermentation of sugars by typhoid and paratyphoid bacilli.

Authors report that glucose may or may not be fermented, lactose never.

Added nutrients: Authors added 1.0% of glucose or lactose.

Reference: Nicolle, Raphael and Debains (1917 p. 379).

575. Robinson and Rettger's Basal Opsine Solution

Constituents:

1. Water	1000.0 cc.
2. Opsine	10.0 g.
3. NaCl	5.0 g.
4. Glycerol	50.0 g.

Preparation:

(1) Dissolve 2, 3 and one of the added nutrients in 1 by heating.

(2) Adjust the reaction—may be used either slightly acid or basic to litmus.

(3) Boil over a flame a few minutes.

(4) Filter and tube.

Sterilization: Sterilize at 12 pounds extra pressure for 15 minutes.

Use: General culture medium. Opsine is a protein free trypsin, erepsin and pepsin digest of certain proteins. The medium supported the growth of some pathogenic forms.

Added nutrients: The authors added 5.0% glycerol or 1.0% glucose.

Reference: Robinson and Rettger (1918 p. 212).

576. Elser and Huntoon's Basal Nährstoff-Heyden Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Nàhrstoff Heyden	10.0 g.
3. NaCl	5.0 g.
4. Litmus (Merck's soln)	5.0 to 7.5 cc.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add 5.0 to 7.5 cc. of a watery solution of Merck's highly sensitized litmus.

(3) Prepare a 10.0% solution of one of the added nutrients in distilled water.

(4) Mix sterile (2) and sterile (3).

(5) Tube in sterile tubes.

(6) Incubate for 3 days to detect accidental contamination.

Sterilization: Sterilize (2) in the usual manner, method not given. Sterilize (3) by heating at 100°C. for 10 minutes.

Use: To study fermentation by meningococci.

Added nutrients: The author added 10.0 g. of one of the following:

glucose
galactose
levulose
lactose
maltose
sucrose
mannitol
dulcitol
inulin
dextrin

Reference: Elser and Huntoon (1909 p. 404).

577. Clark and Lubs' Basal Phosphate Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Witte)	5.0 g.
3. K_2HPO_4 (c.p.)	5.0 g.

Preparation:

(1) Dissolve 2, 3 and one of the added nutrients in 800.0 cc. distilled water by heating for 20 minutes over a steam bath. Stir occasionally.

(2) Filter thru a Schleicher and Schüll No. 588 folded filter.

(3) Cool to 20°C. and make up exactly to 1000.0 cc.

(4) Tube in 10.0 cc. quantities in clean sterile test tubes.

Sterilization: Sterilize by the intermittent method.

Use: Used to study fermentation of sugars.

Added nutrients and variants:

(a) Clark and Lubs added 5.0 g. c.p. glucose.

(b) Levine added 5.0 g. of one of the following materials:

fructose
galactose
maltose
lactose
sucrose
raffinose
mannitol
glycerol
salicin
dextrin

The media were sterilized in the autoclave.

(c) Rogers, Clark and Evans used 10.0 g. Witte's peptone instead of 5.0 g. and added 10.0 g. of one of the following:

glucose
lactose
sucrose
raffinose
melibiose
arabinose
inulin
mannitol
dulcitol
adonitol
glycerol

The media was tubed in 10.0 cc.

quantities and sterilized by the intermittent method in flowing steam.

(d) Levine used Digestive Ferments Co. peptone instead of Witte's, and added 5.0 g. of glucose. The medium was sterilized in the autoclave.

(e) Burton and Rettger added 5.0 g. glucose to the basic solution. The reaction was adjusted to neutral to litmus (pH = 7.0 or +1.0). Method of sterilization not specified.

(f) Kligler used 10.0 g. of peptone instead of 5.0 g. Witte's peptone, added 5.0 g. NaCl, 1.0 g. of glucose and added any organic test material in any desired quantity. The materials used were added to test their antiseptic ability. Sterilize the basic solution with the glucose in the autoclave. Then add one of the materials under aseptic conditions.

(g) Winslow, Rothberg and Parsons added 27.0 cc. of a 0.04% alcoholic solution of brom cresol purple and 5.0 g. of any carbon source or fermentable material. The basic solution was adjusted to pH = 7.4, brom cresol purple added and autoclaved at 15 pounds pressure for 15 minutes. The sterile added nutrient was added under aseptic conditions.

(h) Ayers, Rupp and Mudge used 40.0 g. of Bacto peptone instead of 5.0 g. Witte's peptone and added 2.0 g. glucose. Method of sterilization not specified.

(i) Cunningham autoclaved the K_2HPO_4, peptone and water to 30 pounds pressure, filtered, made up to 1000.0 cc., distributed in 5.0 cc. quantities and autoclaved at 22.5 pounds pressure.

(j) Wilson and Blair made up the basic solution with 5.0 g. glucose in double strength. They used the medium to enrich streptococci in water analyses. Method of sterilization not given. An equal volume of water under investigation was added to the medium.

References: Clark and Lubs (1915 p. 169), Levine (1916 pp. 160, 161), Committee A. P. H. A. (1917 p. 107), Rogers, Clark and Lubs (1918 p. 234), Burton and Rettger (1917 p. 165), Kligler (1918 p. 467), Tanner (1919 p. 47), Winslow, Rothberg and Parsons (1920 p. 151), Levine (1921 p. 117), Ayers, Rupp and Mudge (1921 p. 258), Giltner (1921 p. 383), Cunningham (1924 p. 88), Wilson and Blair (1925 p. 112), Committee A. P. H. A. (1925 p. 111).

578. Grimbert's Basal Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (dry)	20.0 g.
3. $CaCO_3$	

Preparation:

(1) Dissolve 2 and one of the added nutrients in 1.

(2) Add a sufficient quantity of $CaCO_3$.

Sterilization: Not specified.

Use: Determine fermentation of Friedländer's pneumobacillus.

Added nutrients and variants:

(a) The author added 30.0 g. of any fermentable sugar.

(b) Pottevin used 10.0 g. peptone, 12.0 g. of $CaCO_3$ in the basic solution to study the lactic fermentation by lactic acid forming organisms. He added one of the following:

lactose	8.86 g.
sucrose	10.1 g.
maltose	11.4 g.
glucose	10.0 g.
Invert sugar	11.2 g.
galactose	9.2 g.
mannose	8.96 g.
mannitol	10.0 g.
dulcitol	10.0 g.
glycerol	10.0 g.
malic acid	10.0 g.

Reference: Grimbert (1895 p. 843), Pottevin (1898 p. 54).

579. Kendall, Walker and Day's Basal Peptone Salt Solution

Constituents:

1. Redistilled water	1000.0 cc.
2. Peptone (Fairchild)	5.0 g.
3. Na_2HPO_4	2.0 g.
4. NaCl	5.0 g.

Preparation:

(1) Extract 5.0 g. of Fairchild's peptone for 2 weeks with ether, 2 weeks with alcohol, 2 weeks with acetone and 10

days with petroleum ether, respectively, in a Soxhlet extractor, the successive extractions occurring at intervals of about 12 minutes. These extractions were continued for 6 hours a day for 6 days per week.

(2) Dissolve the residue from (1), 3, 4 and one of the added nutrients in 1.

(3) Distribute in 100.0 cc. lots.

Sterilization: Not specified.

Use: To study lipolytic and proteolytic activity of tubercle bacilli. To determine lipolytic activity, the authors placed 1.0 cc. of the bacteria free filtrate of the culture in freshly boiled dist. H_2O, then added 0.25 cc. ethylbutyrate and 0.5 cc. of toluene and incubated at 37°C. The acid increase expressed in terms of N/50 NaOH was a measure of the lipolytic activity.

Added nutrients and variants:

(a) The authors added 10.0 g. glucose or 20.0 g. glycerol.

(b) Kendall, Day and Walker used 15.0 g. of Fairchild's peptone (extracted) and 3.0 g. Na_2HPO_4 in the basic solution. They added 10.0 g. glucose or 30.0 g. glycerol. The solution was used to study the metabolism of tubercle bacilli. Used a alizarin, neutral red and phenolphthalein to study changes in reaction, and Ziehl-Neelsen stain for staining.

(c) The authors used the same basic solution without extracting the peptone, adding 10.0 g. glucose or 30.0 g. glycerol.

References: Kendall, Walker and Day (1914 p. 463), Kendall, Day and Walker (1914 p. 424).

580. Berman and Rettger's Basal Peptone Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte)	2.5 or 5.0 g.
3. NaCl	5.0 g.
4. K_2HPO_4	5.0 to 8.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add one of the combinations listed under added nutrients to (1).

Sterilization: Not specified.

Use: To study nitrogen metabolism of bacteria. In presence of carbohydrates, buffers aided nitrogen metabolism.

Added nutrients: One of the following combinations were added:

(a) glucose, 2.0 to 10.0 g.

(b) lactose, 2.0 to 10.0 g.

(c) glucose, 2.0 to 10.0 g. + beef extract 2.5 g.

(d) lactose, 2.0 to 10.0 g. + beef extract 2.5 g.

Reference: Berman and Rettger (1918 p. 392).

581. Palladin's Basal Peptone Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. Potassium phosphate	3.0 g.
4. $MgSO_4$	1.0 g.
5. $CaCl_2$	1.0 g.
6. $FeCl_2$	trace

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Add one of the added nutrients so that the carbohydrate is present in fourth normal concentration.

(3) Distribute in thin layers in Erlenmeyer flasks.

Sterilization: Not specified.

Use: To study fermentation by *Clorothecium saccharophilum.*

Added nutrients: One of the following were added in fourth normal concentration:

sucrose
mannitol
glucose
raffinose

Reference: Palladin (1903–04 p. 146).

582. Boekhout and Ott de Vries' Basal Peptone Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (2.0%)	20.0 g.
3. Bicalcium phosphate (0.2%)	2.0 g.
4. $CaCl_2$ (concentrated soln.)	5 drops
5. NaCl (0.5%)	5.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and one of the added nutrients in 1.

Sterilization: Not specified.

Use: Cultivation of organisms found in cheese.

Added nutrients: The authors added 0.5% of glucose or galactose.

Reference: Boekhout and Ott de Vries (1918 p. 134).

583. Will's Basal Peptone Salt Solution

Constituents:

1. Distilled water	1000.0	cc.
2. $CaHPO_4$	0.5	g.
3. KH_2PO_4	4.55	g.
4. Peptone (Witte)	20.0	g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Add one of the added nutrients.

Sterilization: Not specified.

Use: Cultivation of non-spore forming yeast.

Added nutrients and variants:

(a) The author added one of the following:

sucrose	5.0%
organic acid, carbohydrate or alcohol	

(b) Grosbüsch added one of the following:

alcohol	5.0%
formic acid	0.1%
acetic acid	0.2%
tartaric acid	0.3%
lactic acid	4.0%
citric acid	8.0%
malic acid	10.0%
succinic acid	11.0%

The amounts of acids given was the largest amount at which *Torula rubefaciens* developed.

Reference: Will (1908 p. 387), (1912 p. 3), Grosbüsch (1920 p. 311).

584. Maassen's Basal Peptone Salt Solution

Constituents:

1. Water	1000.0	cc.
2. Peptone	10.0	g.
3. KH_2PO_4	1.5	g.
4. NaCl	1.0	g.
5. $MgSO_4$	0.3	g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 800.0 cc. of 1.
(2) The reaction of (1) is acid.
(3) Dissolve one tenth the equivalent weight of one of the added nutrients in 200.0 cc. of 1.
(4) Add (3) to (1).
(5) Neutralize and make slightly alkaline to litmus.

Sterilization: Not specified.

Use: To study the utilization of organic acids by organisms. The author used 51 organisms and reported that 41 organisms developed in the presence of malic acid and 38 in the presence of citric and fumaric acids. These acids allowed the development of the greatest number of organisms.

Added nutrients: One tenth equivalent weight of one of the following acids was added:

formic acid
acetic acid
propionic acid
glycolic acid
lactic acid
butyric acid
glyceric acid
"Oralsaure"
malic acid
succinic acid
malonic acid
tartaric acid
fumaric acid
malic acid
"trikarballysaure"
citric acid
aconitic acid
mucic acid
quinic acid
mandelic acid

Reference: Maassen (1895–6 p. 341).

585. Calmette, Massol and Breton's Basal Peptone Salt Solution

Constituents:

1. Water	1000.0	cc.
2. Na_2CO_3	1.0	g.
3. $FeSO_4$	0.04	g.
4. $MgSO_4$	0.05	g.
5. K_2HPO_4	1.05	g.
6. NaCl	8.5	g.
7. Peptone	10.0	g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Add one of the combinations given under added nutrients to (1).

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli. The authors reported the best growth in the presence of both glucose and glycerol.

Added nutrients: One of the following was added:

(a) glycerol 40.0 g.
(b) glucose 10.0 g.
(c) glycerol 40.0 g. + glucose 10.0 g.

Reference: Calmette, Massol and Breton (1909 p. 580).

586. Hansen's Basal Peptone Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	1.0 g.
3. KH_2PO_4	3.0 g.
4. $MgSO_4$	2.0 or 5.0 g.

Preparation: (1) Dissolve 2, 3, 4 and one of the added nutrients in 1. (Use 5.0 g. $MgSO_4$ using maltose and 2.0 g. with glucose.)

Sterilization: Not specified.

Use: Cultivation of *Saccharomyces cerevisiae, Saccharomyces Pastorianus* and a wine yeast.

Added nutrients and variants:

(a) The author added 5.0 g. of maltose or glucose.

(b) Störmer cultivated the causative organism of water rust in flax (*Plectridium pectinovorum*) using 5.0 g. KH_2PO_4, 2.5 g. $MgSO_4$ and 5.0 g. of peptone in the basic solution. One of the following materials was added:

starch	5.0 to 10.0 g.
glucose	5.0 to 10.0 g.
arabinose	5.0 to 10.0 g.
dextrin	5.0 to 10.0 g.
galactose	5.0 to 10.0 g.
pectin substances	2.5 to 5.0 g.

All the solutions were sterilized in the autoclave except the one containing pectin. Sterilize this solution at 100°C. Incubate under anaerobic conditions, using pyrogallic acid and KOH.

(c) Buromsky studied the effect of organic acids upon the growth of yeast, *Saccharomyces ellipsoid,* and *Saccharomyces pastorian.* He used 0.5 g. $MgSO_4$, 1.0 g. KH_2PO and 10.0 g. peptone in the basic solution and added one of the following:

citric acid	1.0%
tartaric acid	1.0%
malic acid	1.0%
quinic acid	0.5%
succinic acid	1.0%
glycerol	1.0%
mannitol	1.0%

He reported no growth using tartaric acid. Citric acid also inhibited most yeast.

References: Hansen (1899 p. 6), Störmer (1904 p. 177), Buromsky (1914 p. 532).

587. Stoklasa and Vitek's Basal Peptone Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Na_2HPO_4	0.25 g.
3. K_2SO_4	0.2 g.
4. Na_2CO_3	0.25 g.
5. $FePO_4$	0.2 g.
6. $MgCl_2$	0.1 g.
7. $CaCl_2$	0.05 g.
8. Peptone	0.1 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

(2) Distribute in 100.0 cc. lots.

(3) Add one of the added nutrients to each flask. (Neutralize and make the acids slightly alkaline by the addition of Na_2CO_3).

(4) Add 2.0 g. $CaCO_3$ to each flask.

Sterilization: Method not given.

Use: To study nitrogen assimilation by *Bacillus megatherium* (Alinit bacteria). Authors reported that organisms showed greater development in medium containing organic acids as a source of carbon than d-glucose. Greatest development took place with saccharose, l-arabinose and l-xylose. No growth took place using d-laevulose or d-galactose. Lactic acid is the best suited acid for *B. megatherium* development.

Added nutrients: The authors added 10.0% of one of the following:

d-glucose
d-laevulose
d-galactose
d-xylose
l-arabinose
saccharose
maltose
tartaric acid
citric acid
malic acid
lactic acid
butyric acid

Reference: Stoklasa and Vitek (1901 p. 268).

588. Vierling's Basal Peptone Salt Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.1 g.
5. $FeCl_3$	trace
6. NaCl	trace
7. Peptone	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Add one of the combinations given under added nutrients.

(3) Distribute in 5.0 cc. quantities in tubes.

Sterilization: Not specified.

Use: To study nitrate reduction by mycobacteria. To test for nitrite production add a 2.0% acidified KI solution to the tube. A blue color will develop in the presence of nitrites. The presence of nitrite may be detected also by dissolving 0.1 g. napthylamine in 150.0 g. dilute acetic acid, and 0.5 g. of sulfanilic acid in 150.0 g. dilute acetic acid. Mix 1.0 cc. of each solution and heat to boiling. Add 1.0 cc. of the mixture to the culture. If nitrite be present a red color will be found.

Added nutrients and variants:

(a) Starch 10.0 g. + KNO_3 5.0 g.
(b) Peptone 20.0 g. in the basic solution and added 10.0 g. of starch. This medium was used to study amylase production by mycobacteria. No reducing sugars were found to be present using Fehling's solution for the test.
(c) Glucose 10.0 g. This solution was used to study catalase production by mycobacteria. Catalase was produced. A 14 day culture quickly decomposed 1.0 cc. of a 15.0 % H_2O_2 solution, of oxygen with the libera- of oxygen.

Reference: Vierling (1920 pp. 201, 204, 207).

SUBGROUP I-C. SECTION 9

Complete or basal liquid media containing peptone or similar digests as sole sources of nitrogen; additional organic carbon supplied.

A_1.* Carbohydrates present.
B_1. Carbohydrate only added.
C_1. Monosaccharides added.
D_1. Levulose added.
Müller, Thurgau and Osterwälder's Levulose Peptone Solution........ 589
D_2. Glucose added.
E_1. Containing phosphates.
F_1. Containing salts of monovalent cations only.
Kligler's Glucose Peptone Solution.. 590
Löhnis' Glucose Peptone Solution... 591
F_2. Containing salts of mono and divalent cations.
Mueller's Glucose Peptone Salt Solution.............................. 592
Schukow's Basal Glucose Peptone Salt Solution.................... 593
Henneberg's Glucose Peptone Salt Solution.......................... 594
E_2. Not containing phosphates.
F_1. Salts of monovalent cations only added.
Harvey's Glucose Peptone Solution.. 595
Eijkman's Glucose Peptone Solution. 596
Matzuschita's Glucose Peptone Solution.............................. 597
F_2. Salts of divalent cations with or without other cations added.
Gosio's Glucose Peptone Solution... 598
Sears' Glucose Peptone Solution.... 599
Ringer's Solution Broth (Park, Williams and Krumwiede)............ 600
C_2.* Disaccharides added.
D_1. Lactose employed.
E_1. Inorganic salts not added.
Grimbert's Lactose Peptone Solution (Robin)...................... 601
Levine's Lactose Peptone Solution.. 602
Wagner and Monfort's Lactose Peptone Solution.................... 603
Baginsky's Lactose Peptone Solution. 604
E_2. Inorganic salts added.
F_1. Indicators added.
Robin's Lactose Peptone Solution... 605
Klein's Lactose Peptone Solution.... 606
Harvey's Lactose Peptone Solution.. 607
Olszewski and Köhler's Lactose Peptone Solution.................... 608
Bacto Neutral Red Medium (Dehydrated)......................... 609
F_2. No indicators added.
Fischer and Andersen's Lactose Peptone Solution.................... 610
Baginsky's Lactose Peptone Salt Solution.......................... 611
D_2. Sucrose employed.
E_1. Containing salts of monovalent cations only.
Bendick's Phenolphthalein Sucrose Solution......................... 612
Smith's Sucrose Peptone Solution (Owen).......................... 613
Briaudat's Sucrose Peptone Solution. 614
Bokorny's Sucrose Peptone Solution. 615
E_2. Containing salts of mono and divalent cations.
Mutchler's Sucrose Peptone Solution. 616
Waksman's Sucrose Peptone Solution. 617
Zikes' Sucrose Peptone Solution..... 618
Behrens' Basal Sucrose Peptone Solution.............................. 619

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* See next page for A_2 and B_2.

*See C_3 next page.

Bokorny's Sucrose Somatose Solution. 620

E_3. Containing salts of mono, di and trivalent cations.

Trommsdorff's Sucrose Peptone Solution 621

Vierling's Sucrose Peptone Solution. 622

C_3. Polysaccharides added.

Baginsky's Starch Peptone Solution. 623

Dumas' Glycogen Peptone Solution. 624

Kodama and Takeda's Starch Peptone Solution 625

Khouvine's Cellulose Peptone Solution 626

Vierling's Cellulose Peptone Solution. 627

B_2. Carbohydrates added together with other organic carbon compounds.

C_1. Alcohols employed.

Matzuschita's Pyrogallic Acid Bouillon 628

Bezançon's Glycerol Peptone Solution 629

C_2. Organic acids employed.

Behrens' Tartrate Peptone Solution. 630

Dombrowski's Glucose Peptone Solution 631

Zumstein's Citric Acid Peptone Solution (Killer) 632

Seitz's Double Sugar Peptone Solution (Harvey) 633

Müller, Thurgau and Osterwälder's Malic Acid Peptone Solution 634

Ayers, Rupp and Johnson's Glucose Peptone Solution 635

A_2. Carbohydrates not present.

B_1. Additional organic carbon supplied as alcohols.

Marmier's Glycerol Protease Solution 636

Frankland and Fox's Glycerol Peptone Solution (Owen) 637

Harvey's Glycerol Peptone Solution 638

Waksman's Basal Glycerol Peptone Solution 639

Capaldi and Proskauer's Mannitol Peptone Solution 640

B_2. Additional organic carbon supplied as acids or their salts.

Omeliansky's Formate Peptone Solution 641

Voges and Proskauer's Basal Citrate Peptone Solution 642

Behrens' Basal Citrate Acid Peptone Solution 643

Freudenreich and Jensen's Lactate Peptone Solution 644

Baginsky's Lactate Peptone Solution 645

Harvey's Tartrate Peptone Solution. 646

Harrison and Vanderleck's Aesculin Peptone Solution 647

589. Müller, Thurgau and Osterwälder's Levulose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Levulose	10.0 g.
3. Peptone	10.0 g.
4. K_2HPO_4	0.5 g.
5. $MgSO_4$	0.1 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of bacteria from wine and fruit wine, *Micrococcus malolacticus*.

Reference: Müller, Thurgau and Osterwälder (1912-13 p. 155).

590. Kligler's Glucose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (0.5%)	5.0 g.
3. Glucose (0.5%)	5.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: To study metabolism of colon-typhoid group.

Variants: Kligler gives the following solutions:

(a) Glucose 0.5% + 1.0% peptone.
(b) Glucose 0.5% + 1.5% peptone.
(c) Glucose 0.5% + 0.5% peptone + 0.5% Na_2HPO_4.
(d) Glucose 0.5% + 1.0% peptone + 0.5% Na_2HPO_4.
(e) Glucose 0.5% + 1.5% peptone + 0.5% Na_2HPO_4.
(f) Glucose 0.3% + 1.0% peptone + 0.2% Na_2HPO_4.
(g) Glucose 0.3% + 1.0% peptone + 0.3% Na_2HPO_4.
(h) Glucose 0.3% + 1.0% peptone + 0.4% Na_2HPO_4.
(i) Glucose 0.4% + 1.0% peptone + 0.2% Na_2HPO_4.
(j) Glucose 0.4% + 1.0% peptone + 0.3% Na_2HPO_4.
(k) Glucose 0.4% + 1.0% peptone + 0.4% Na_2HPO_4.

Reference: Kligler (1916 pp. 668-669).

591. Löhnis' Glucose Peptone Solution

Constituents:

1. Water tap	1000.0 cc.
2. Glucose (4.0%)	40.0 g.
3. Peptone (0.1%)	1.0 g.
4. K_2HPO_4 (0.05%)	0.5 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of aerobacter and amylobacter. The medium was inoculated with 10.0% soil. Similar media were used to study the fermentation of glucose, for methyl red test.

Variants:

(a) Rogers, Clark and Evans prepared the medium as follows:
  (1) Dissolve 10.0 g. glucose, Merck's highest quality, 10.0 g. Witte's peptone and 5.0 g. K_2HPO_4 in 800.0 cc. distilled water, heating over the steam for 20 minutes, and occasionally stirring.
  (2) Filter thru a Schleicher and Schull No. 588 folded filter.
  (3) Allow the filtrate to cool to 20°C. and make up to 1000.0 cc.
  (4) Sterilize in the steamer on each of 3 successive days.

(b) Clark and Lubs used the same medium as in variant (a), but omitted the K_2HPO_4 with or without the addition of H_3PO_4 until the solution is 0.075 molar NaOH.

(c) Clark and Lubs used the same medium as in variant (a) but used 5.0 to 8.0 g. glucose instead of 10.0 g.

(d) Bunker, Tucker and Green gave the same formula as variant (a) but used 5.0 g. glucose and 5.0 g. peptone and omitted the phosphate.

References: Löhnis (1913 p. 104), Rogers, Clark and Evans (1914 p. 103), Clark and Lubs (1915 pp. 162, 163), Bunker, Tucker and Green (1918 p. 493), Committee American Public Health Association (1923 p. 107), (1925 p. 111).

592. Mueller's Glucose Peptone Salt Solution

Constituents:

1. Water	2000.0 cc.
2. Peptone	20.0 g.
3. NaCl	10.0 g.
4. $MgSO_4$	0.4 g.
5. $CaCl_2$	0.2 g.
6. K_2HPO_4	2.0 g.
7. Glucose	2.0 g.
8. Phenol red (0.02% soln.)	2.0 g.

Preparation:
(1) Dissolve 2 in 1000.0 cc. of 1.
(2) Dissolve 3, 4, 5, 6, 7 and 8 in 1000.0 cc. of 1.
(3) Mix (1) and (2).
(4) Add NaOH until the medium is red (pH = 7.8).

Sterilization: Sterilize in autoclave at 10 pounds for 10 minutes.

Use: To study the constituents essential for the growth of streptococci and pneumococci. The author reported this as a poor medium.

Reference: Mueller (1922 pp. 317, 325).

593. Schukow's Basal Glucose Peptone Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. Dextrose	100.0 g.
4. Potassium phosphate	5.0 g.
5. $MgSO_4$	0.5 g.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Add 9.0 to 10.0% of one of the added nutrients to (1).
(3) Adjustment of reaction not specified.
(4) Distribute into fermentation flasks (sealed with H_2SO_4).

Sterilization: Sterilize in a steamer.

Use: To study the utilization of acids by yeast. After 85 days 0.124 g. of tartaric acid and 0.27 g. of malic acid were used. Acid determined by titration using litmus as an indicator.

Added nutrients: The author added 9.0 or 10.0% of malic or tartaric acid.

Reference: Schukow (1896 p. 608).

594. Henneberg's Glucose Peptone Salt Solution

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4 (0.8%)	8.0 g.
3. $MgSO_4$ (0.1%)	1.0 g.
4. Glucose (5.0%)	50.0 g.
5. Peptone (1.0 to 5.0%)	10.0 to 50.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of lactic acid bacteria.
Reference: Henneberg (1903 p. 8).

595. Harvey's Glucose Peptone Solution

Constituents:

1. Distilled water	960.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.
4. Glucose	10.0 g.
5. Litmus solution	40.0 cc.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Filter sterile (1) and adjust the reaction if necessary.
(3) Dissolve 10.0 g. glucose in 40.0 cc. of litmus solution.
(4) Mix (2) and (3).
(5) Filter thru paper.
(6) Steam 10 minutes.
(7) Tube sterile (6) into sterile test tubes.

Sterilization: Sterilize (1) in the autoclave. Sterilize (6) by filtering thru a candle. Incubate to test sterility before use.

Use: General culture medium.

Reference: Harvey (1921–22 p. 109).

596. Eijkman's Glucose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose (10.0%)	100.0 g.
3. Peptone (10.0%)	100.0 g.
4. NaCl (5.0%)	50.0 g.

Preparation: (1) Prepare a solution containing 10.0% glucose, 10.0% peptone and 5.0% NaCl.

Sterilization: Not specified.

Use: Water analysis and detection of *B. coli*. The water under investigation is placed into fermentation flasks and $\frac{1}{3}$ of its volume of the medium is added. The presence of uniform turbidity and gas formation after 24 hours at 46°C. indicates the presence of *B. coli*. Indol may be formed after 48 hours.

Variants:

(a) Truche and Cotoni enriched pneumococci in the following solution:

1. Water	1000.0 cc.
2. Peptone (Chapoteaut)	40.0 g.
3. NaCl	5.0 g.
4. Glucose	2.0 g.

Chapoteaut's peptone cannot be replaced by Witte's or Defresne's. They specified that one-third part ascitic fluid might be added. Pneumococci grew very well without the ascitic fluid however.

(b) Stewart and Stitt, determined the Voges Proskauer reaction using 5.0 g. NaCl, 20.0 g. glucose and 10.0 g. Witte's peptone per liter.

(c) Meyers studied H_2S production using 30.0 g. of Witte's, Difco or Fairchild's peptone, 5.0 g. glucose, 5.0 g. NaCl, and 0.0 or 5.0 g. sucrose per liter.

(d) Klimmer used 10.0 g. peptone, 5.0 g. NaCl and 10.0 g. of glucose per liter.

References: Eijkman (1904 p. 745), Truche and Cotoni (1911 p. 480), Stewart (1917–18 p. 294), Myers (1920 p. 242), Stitt (1923 p. 36), Klimmer (1923 p. 221).

597. Matzuschita's Glucose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Meat peptone (Koch)	10.0 g.
3. Gum arabic	50.0 to 300.0 g.
4. NaCl	5.0 g.
5. Glucose	20.0 g.

Preparation:

(1) Dissolve 2 in 1.
(2) Dissolve 3, 4 and 5 in (1).
(3) Neutralize (indicator not specified).

Sterilization: Sterilize in the steamer on from 2 to 5 successive days for 15 to 30 minutes. Incubate for 2 days at 37°C. to test sterility.

Use: Cultivation of spore forming bacilli. *Clostridium butyricum, Bacillus oedematis maligni, Bacillus anthracis symptomatici, Bacillus sporogenes, Bacillus botulinus.*

Variants: The author used 1.0 to 3.0% gum tragacanth instead of 50 to 300.0 g. gum arabic.

Reference: Matzuschita (1902 p. 287).

598. Gosio's Glucose Peptone Solution

Constituents:

1. Distilled water	3000.0 cc.
2. Peptone	30.0 g.
3. Dextrose	138.0 to 150.0 g.
4. Na_2CO_3	1.6 g.
5. $CaCO_3$	75.0 g.

Preparation:

(1) Dissolve 2 and 4 in part of 1.

(2) Dissolve 3 in the remainder of (1).

(3) Mix sterile (1) and (2) when cool. The addition of $CaCO_3$ not specified.

Sterilization: Method not given.

Use: Lactic acid production by vibrio.

Variants: The author used the following solution with 30.0 cc. normal soda solution to study fermentation by *Vibrio cholera asiaticae.*

1. Water	3000.0 cc.
2. Peptone (Witte)	30.0 g.
3. Glucose	150.0 g.
4. $CaCO_3$	75.0 g.

Reference: Gosio (1894 p. 116, 2).

599. Sears' Glucose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	20.0 g.
3. Glucose	5.0 g.
4. NaCl	5.0 g.
5. $CaCO_3$	

Preparation:

(1) Dissolve 2 and 4 in 1.

(2) Divide in two parts.

(3) To one part of (2) add 1.0% (5.0 g.) of pure glucose and a little $CaCO_3$.

(4) Distribute both portions in 200.0 cc. lots into 300.0 cc. flasks.

Sterilization: Sterilize in autoclave for 10 minutes at 15 pounds pressure.

Use: To study nitrogen metabolism. Ammonia determined by Folin's aeration method, amino acid by Van Slyke's micro method and total N by Kjeldahl-Gunning-Arnold method.

Reference: Sears (1916 p. 118).

600. Ringer's Solution Broth (Park, Williams and Krumwiede)

Constituents:

1. Water	1000.0 cc.
2. NaCl	10.0 g.
3. KCl	0.2 g.
4. $CaCl_2$	0.2 g.
5. Sodium bicarbonate	0.1 g.
6. Glucose	1.0 g.
7. Peptone (1.0 or 2.0%)	10.0 or 20.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: General culture medium.

Reference: Park, Williams and Krumwiede (1924 p. 122).

601. Grimbert's Lactose Peptone Solution (Robin)

Constituents:

1. Distilled water	1000.0 cc.
2. Lactose (pure)	20.0 g.
3. Peptone	5.0 g.
4. Litmus	

Preparation:

(1) Boil 2 and 3 in 1, until solution is complete.

(2) Add lime water to neutralize.

(3) Filter and readjust the reaction to neutral to litmus if necessary.

(4) The medium is tubed before sterilization if heated, but tubed in sterile tubes if filtered to sterilize.

(5) Add sufficient sterile litmus solution to each tube to give color.

Sterilization: Sterilize (3) by heating at 110° for 15 minutes or better by filtering thru a clay candle. Sterilize the litmus solution in the autoclave.

Use: Differentiation of colon-typhoid group. Colon bacilli gave an acid reaction, typhoid bacilli did not change the reaction.

Reference: Robin (1901 p. 915).

602. Levine's Lactose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (1.0%)	10.0 g.
3. Lactose (0.5%)	5.0 g.
4. Brilliant green	

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add varying concentrations of brilliant green.

Sterilization: Not specified.

Use: Differentiation of *Bact. coli* and *Bact. aerogenes.* Author reported that *Bact. aerogenes* grew luxuriantly with 1–750,000 brilliant green. *Bact. coli* did not grow at this dye concentration. If the peptone content is decreased to 0.5% the antiseptic action of brilliant green is very markedly increased for both organisms.

Variants: Used 0.5% peptone instead of 1.0%.

Reference: Levine (1911 p. 22).

603. Wagner and Monfort's Lactose Peptone Solution

Constituents:

1. Water	1000.0 cc.

2. Peptone (2.0%)	20.0 g.
3. Lactose (0.2%)	2.0 g.
4. Gentian violet (0.001%)	0.01 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) The reaction is between pH 7.0 and 7.6.

(3) Tube and plug with cotton.

Sterilization: Pasteurize at 70 to 80°C. for 15 minutes.

Use: Presumptive test for *Bact. coli* in water analysis. Author reported that the medium gave as good results as when the usual 1.0 or 0.5% lactose was used. 0.001 to 0.00033% of gentian violet may be used.

Variants: Used 0.001 to 0.00033% gentian violet instead of 0.01%.

Reference: Wagner and Monfort (1921 p. 207).

604. Baginsky's Lactose Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Lactose	300.0 g.
3. Peptone	

Preparation:

(1) Distribute distilled water in 25.0 cc. quantities in small flasks that have been sterilized at 140°C.

(2) Add 5.0 g. of lactose and a small amount (exact amount not given) of peptone to each flask.

Sterilization: Sterilize in flowing steam on 3 successive days for ½ to 1 hour.

Use: To study fermentation by *B. lactis*.

Reference: Baginsky (1888 pp. 434–462).

605. Robin's Lactose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Collas)	20.0 g.
3. Sodium phosphate	0.2 g.
4. NaCl	2.0 g.
5. Soluble blue (1.0% soln.)	4.0 cc.
6. Lactose	80.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1 by boiling.

(2) Add 4.0 cc. of 1.0% aqueous solution of soluble blue ("blue soluble") to boiling (1).

(3) Add sufficient tenth normal KOH solution to decolorize (2).

(4) Add 80.0 g. of lactose to (3).

(5) Filter.

(6) Distribute in 10.0 cc. lots in Pasteur flasks.

Sterilization: Sterilize in the autoclave at 105° for 15 minutes.

Use: Differentiation of colon and typhoid groups. Author reported that coli strains produced a blue colored medium, typhoid cultures clouded the medium but gave no change in color.

Reference: Robin (1897 p. 49).

606. Klein's Lactose Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone	2.5 g.
3. Na_2HPO_4	2.5 g.
4. NaCl	5.0 g.
5. Lactose	5.0 g.
6. Azolitmin	0.25 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Study of mutation in colon group.

Reference: Klein (1913 p. 100).

607. Harvey's Lactose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.
4. Lactose (1.0%)	10.0 g.
5. Neutral red (sat. soln.) (1.0%)	10.0 cc.

Preparation:

(1) Dissolve 2 and 3 in 1 by boiling.

(2) Dissolve 4 and 5 in (1) by heating.

(3) Filter.

(4) Distribute in test tubes containing fermentation tubes.

Sterilization: Sterilize on each of 3 successive days at 100°C.

Use: General culture medium. Park, Williams and Krumwiede used the medium in the examination of water, sewage and shellfish.

References: Harvey (1921–22 p. 101), Park, Williams and Krumwiede (1924 p. 132).

608. Olszewski and Köhler's Lactose Peptone Solution

Constituents:

1. Water	600.0 cc.
2. Peptone	75.0 g.
3. NaCl	50.0 g.

4. Lactose	75.0 g.
5. Litmus solution	500.0 cc.

Preparation:

(1) Dissolve 75.0 g. peptone and 50.0 g. NaCl in 600.0 cc. water.

(2) Filter.

(3) Dissolve 75.0 g. lactose in 500.0 cc. litmus solution, made according to Kubel and Tiemann with Kahlbaum's litmus (method or reference not given).

(4) Boil (3) for 15 minutes.

(5) Filter.

(6) Add NaOH or soda solution to neutralize if necessary.

(7) Mix sterile (2) and (6) when ready for use.

(8) Tube.

Sterilization: Method of sterilization of (2), (6) or (8) not given.

Use: Presumptive test for *B. coli* in water analysis. Ten parts water under investigation were added to one part of medium. *B. coli* turned the medium red.

Reference: Olszewski and Köhler (1922 p. 305).

609. Bacto Neutral Red Medium (Dehydrated)

Constituents:

1. Distilled water	
2. Peptone, Bacto	30.0 g.
3. Lactose, Bacto	6.0 g.
4. KCl	8.0 g.
5. Neutral red	0.08 g.

Preparation:

(1) Dissolve 44.0 g. of Bacto neutral red medium in 1000.0 cc. of distilled water.

(2) If sterilized 20 minutes at 15 pounds pressure pH = 6.7.

Sterilization: Sterilize in the usual manner.

Reference: Digestive Ferments Co. (1925 p. 13).

610. Fischer and Andersen's Lactose Peptone Solution

Constituents:

1. Water (96.5%)	965.0 cc.
2. Peptone (Witte) (1.0%)	10.0 g.
3. Lactose (2.0%)	20.0 g.
4. NaCl (0.5%)	5.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Adjustment of reaction not given.

(3) Distribute in 50.0 cc. flasks.

Sterilization: Not specified.

Use: To study acid production by *Bacterium coli*.

Reference: Fischer and Andersen (1912 p. 289).

611. Baginsky's Lactose Peptone Salt Solution

Constituents:

1. Distilled water	750.0 cc.
2. Lactose	36.0 g.
3. Peptone (siccum)	8.0 g.
4. K_2HPO_4	1.6 g.
5. $CaCl_2$	0.15 g.
6. $MgSO_4$	0.3 g.
7. $CaCO_3$	0.02 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Distribute into sterile Erlenmeyer flasks.

Sterilization: Sterilize in flowing steam for 4 days for $\frac{1}{2}$ to 1 hour.

Use: To study fermentation by *B. lactis*.

Reference: Baginsky (1888 pp. 434–462).

612. Bendick's Phenolphthalein Sucrose Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.
4. Na_2CO_3	1.0 g.
5. Sucrose	5.0 g.
6. Phenolphthalein (Sat. 50.0% alc. soln.)	5.0 cc.

Preparation:

(1) Dissolve 2 and 3 in 1 by heating over a free flame.

(2) Add Na_2CO_3 to neutralize to phenolphthalein.

(3) Add 1.0 g. of Na_2CO_3 to (2), and boil over a free flame.

(4) Filter thru a double layer of filter paper.

(5) Add 5.0 g. sucrose and 5.0 cc. of a 50.0% saturated alcoholic solution of phenolphthalein to (4).

(6) Medium should be moderately deep red.

Sterilization: Sterilize in the Arnold for 20 minutes if the medium is to be used at once. If not to be used at once, sterilize in the Arnold for 15 minutes on each of 3 successive days.

Use: Detection of the cholera vibrio. The cholera vibrio decolorized the medium.

References: Bendick (1911 p. 907), (1912 p. 536), Park, Williams and Krumwiede (1924 p. 130), Abbott (1921 p. 570).

613. Smith's Sucrose Peptone Solution (Owen)

Constituents:

1. Water	1000.0 cc.
2. Sucrose	100.0 or 200.0 g.
3. KCl	5.0 g.
4. Na_2HPO_4	2.0 g.
5. Peptone	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Distribute in 200.0 cc. quantities in 300.0 cc. Erlenmeyer flasks.

(3) After final sterilization adjust to pH values varying from pH = 6.7 to 8.5 by the addition of normal NaOH and H_2SO_4. The medium should still be warm.

Sterilization: Sterilize on each of 3 successive days for 30 minutes.

Use: To study gum formation. Author reported the optimum pH between 6.7 and 7.0.

Variants: Invertase may be added to invert the sugar. Levan formation was not aided.

Reference: Owen (1923 pp. 423, 431).

614. Breaudat's Sucrose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Chapoteaut)	10.0 g.
3. Sucrose	30.0 g.
4. K_2CO_3	4.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Distribute in two liter flasks.

Sterilization: Method not given.

Use: Acetone production by *Bacillus violacius acetonicus*.

Reference: Breaudat (1906 p. 877).

615. Bokorny's Sucrose Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Sucrose	200.0 g.
3. Peptone (meat)	5.0 g.
4. KH_2PO_4	2.0 g.
5. $MgSO_4$	1.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: To study the effect of constituents of the medium on the growth of yeast.

Variants: Bokorny gives the following variants:

(a) 50.0 g. of sucrose instead of 200.0 g.

(b) 2.0 g. of $MgSO_4$ instead of 1.0 g. and added 0.1 g. H_2SO_4.

(c) Added 0.1 g. (or more) of H_3PO_4 or lactic acid.

(d) Added 0.1 g. of HF or formaldehyde.

(e) Added 1.0 g. of formaldehyde, NaCl, $CaCl_2$ or caffeine.

(f) Used 1.0 g. peptone, 1.0 or 10.0 g. sucrose, 1.0 g. KH_2PO_4 and 0.5 g. $MgSO_4$ instead of amounts indicated in original medium and added 0.5 g. $CaCl_2$ and a trace of $FeCl_2$.

(g) Used 1.0 g. peptone, 1.0 or 10.0 g. sucrose 1.0 g. KH_2PO_4 instead of amounts indicated, omitted the $MgSO_4$ and added 0.5 g. K_2SO_4, a trace of $FeCl_2$ and 0.5 g. of KCl or $CaCl_2$.

(h) Used 50.0 g. sucrose, 10.0 g. peptone, 5.0 g. KH_2PO_4 or NaH_2PO_4 and 5.0 g. of $MgSO_4$ instead of the amounts given in the original medium and added 0.0 or 5.0 g. Rb_2SO_4.

(i) Used 100.0 g. sucrose, 25.0 g. peptone, 4.0 g. KH_2PO_4 instead of given amounts.

(j) Used 1.0 g. peptone, 10.0 g. sucrose, 1.0 g. KH_2PO_4, and 0.5 g. $MgSO_4$ instead of the given amounts, and added a trace of iron chloride with 0.5 g. of $CaCl_2$, 0.5 g. KCl or 0.5 g. $CaCl_2 + 0.5$ g. K_2SO_4.

(k) Used 50.0 g. sucrose, 1.0 g. peptone, 0.5 g. $MgSO_4$, omitted the KH_2PO_4 from the original medium and added a trace of iron chloride, a trace of $CaCl_2$ with 1.0 g. KH_2PO_4, 0.1 g. Rb_2SO_4 + 1.0 g. NaH_2PO_4 or 1.0 g. NaH_2PO_4.

References: Bokorny (1902 p. 58), (July 1903), (1903–04 p. 16), (1903–04 p. 20), (1911 p. 182), (1912 p. 128), (1912 p. 128).

616. Mutchler's Sucrose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	20.0 g

3. KH_2PO_4	100.0 g.
4. $MgSO_4$	0.2 g.
5. Calcium phosphate	0.2 g.
6. Sucrose	150.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Flask.

Sterilization: Sterilize in an autoclave on successive days with temperatures of 115° to 120°C. (15 to 18 pounds pressure).

Use: Cultivation of yeast (*Saccharomyces cereviseae*). Author reported a more rapid growth than in beer wort.

Reference: Mutchler (1905–06 p. 34).

617. Waksman's Sucrose Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone	20.0 g.
3. K_2HPO_4	1.0 g.
4. KCl	0.5 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.01 g.
7. Cane sugar	30.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Distribute (1) in 100.0 cc. portions in 200.0 cc. Erlenmeyer flasks.

Sterilization: Sterilize at 15 pounds for 15 minutes.

Use: Cultivation of soil actinomyces and fungi.

Variants: Author used 1.0, 3.0, 5.0 or 20.0% sucrose.

Reference: Waksman (1918 p. 510).

618. Zikes' Sucrose Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Sucrose	100.0 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4$	0.3 g.
5. Peptone	2.5 g.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.
(2) pH = 6.0603, CH = 8.7×10^{-7}.

Sterilization: Not specified.

Use: Volutin formation by *Saccharomyces Frohberg*, wine yeasts, *Saccharomyces anamensis*, *Saccharomyces ilicis Saccharomyces Will (Bajanus)*, *Pichia membranaefaciens*, *Cholera mycoderma*, *Monilia candida*, *Oidium lactic*, *Endomyces Magnusi*.

Reference: Zikes (1922 p. 29).

619. Behrens' Basal Sucrose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Cane sugar	100.0 g.
3. Peptone	5.0 g.
4. $MgSO_4$	1.0 g.
5. K_2HPO_4	2.0 g.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Distribute in 100.0 cc. lots.
(3) Add one of the added nutrients as indicated below.

Sterilization: Method not given.

Use: To study the effect of organic acids on growth of *Penicillium glaucum*, *Pencillium luteum* and *Botrytis* spores.

Added nutrients: The author added one of the following to each lot:

malic acid	0.5, 1.0, 1.5, 2.0 or 5.0 g.
phloroglucine	0.5 or 1.0 g.
hydrochinon	0.5 or 1.0 g.
tartaric acid	7.35 g.
citric acid	6.86 g.

Reference: Behrens (1898 pp. 644, 739).

620. Bokorny's Sucrose Somatose Solution

Constituents:

1. Water	1000.0 cc.
2. Somatose	2.5 g.
3. Sucrose	50.0 g.
4. KH_2PO_4	2.0 g.
5. $MgSO_4$	1.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of yeast.

Reference: Bokorny (1917 p. 364).

621. Trommsdorff's Sucrose Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $MgSO_4$	0.05 g.
3. $CaCO_4$	0.05 g.
4. K_2HPO_4	0.1 g.
5. NaCl	0.1 g.
6. $FeCl_3$	0.001 g.
7. Peptone (0.1%) (1.0 g. or 0.5%)	5.0 g.
8. Sucrose (0.5%)	5.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: Cultivation of *Leptomitus*. Growth very good on this medium. Better growth using 0.5% peptone than 0.1%.

Reference: Trommsdorff (1918 p. 65).

622. Vierling's Sucrose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.1 g.
5. $FeCl_3$	trace
6. NaCl	trace
7. Peptone	10.0 g.
8. Sucrose	10.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: To determine invertase production by mycobacteria. Test for reducing sugars with Fehling's solution. Very small amounts of sugar if any were reduced.

Reference: Vierling (1920 p. 206).

623. Baginsky's Starch Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Starch	10.0 g.
3. Potassium phosphate	0.1 g.
4. $MgSO_4$	0.02 g.
5. Peptone	5.0 g.
6. $CaCl_2$	0.02 g.
7. $CaCO_3$	0.02 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Distribute into sterile flasks.

Sterilization: Steam to sterilize.

Use: To study fermentation.

Reference: Baginsky (1888 pp. 434–462).

624. Dumas' Glycogen Peptone Solution

Constituents:

1. Distilled water	100.0 cc.
2. Peptone (Chapoteaut)	1.0 g.
3. NaCl	0.5 g.
4. Glycogen	0.5 g.
5. Litmus	

Preparation:

(1) Dissolve 2 in 1 by heating.
(2) Dissolve 3 in (1).
(3) Neutralize to litmus.
(4) Add 5.0 cc. of normal soda per liter (0.5 cc. for 100.0 cc.).
(5) Boil slowly for several minutes.
(6) Filter thru wet paper.
(7) Add 4 to (6) and shake a few minutes.
(8) Add drop by drop enough litmus solution to give the desired color.

Sterilization: Sterilize for 15 minutes at 110°C.

Use: To study the fermentation of glycogen by the cholera vibrio. Dumas reported that glycogen was fermented, the medium was turned red, but gas was not formed at the end of 18 or 24 hours.

Reference: Dumas (1919 p. 547).

625. Kodama and Takeda's Starch Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte)	10.0 g.
3. Potato starch	5.0 g.
4. 10.0% soda solution	20.0 cc.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Distribute into sterile tubes.

Sterilization: Sterilize on 2 or 3 successive days (method not given).

Use: To determine digestion of starch. The author added 5.0 cc. of Lugol's solution (1.0 g. iodine 2.0 g. KI in 100.0 cc.) water to the cultures. After 24 hours the starch had nearly disappeared from the cholera cultures and only a yellow color appeared when the iodine solution was added. The cholera-like cultures used less starch and the remaining cultures used no starch at all as was indicated by the amount of starch left in the bottom of the tube and the color change when iodine solution was added.

Reference: Kodama and Takeda (1922 p. 514).

626. Khouvine's Cellulose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. NaCl	1.0 g.
4. Peptone (Pancreatic)	1.0 g.
5. Cellulose	

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Reaction is neutral.
(3) Distribute in 5.0 cc. lots in tubes.

(4) Add 1 square centimeter of cellulose in the form of Berzelius filter paper or a little cellulose precipitated after having been dissolved in Schweitzer's reagent to each tube.

Sterilization: Sterilize for 15 minutes at 110°C.

Use: Isolation and enrichment of *B. cellulose dissolvens*

Variants: The author gives the following variants:

(a) Addition of 2.0 g. glucose.

(b) Used 0.5 g. K_2HPO_4 instead of 1.0 g., added 0.5 g. Na_2HPO_4, and 2.0 g. of $CaCO_3$.

(c) Same as (b) with 2.0 g. of glucose.

(d) Used traces of NaCl instead of 1.0 g., added 0.5 g. $MgSO_4$, traces of $CaCO_3$ and some gum arabic (amount not given).

(e) Same as (d) with 2.0 g. glucose.

Reference: Khouvine (1923 p. 713).

627. Vierling's Cellulose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (3.0%)	30.0 g.
3. $CaCO_3$ (0.1%)	1.0 g.
4. Filter paper	

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Place strips of filter paper in flasks.

(3) Pour (1) into (2).

(4) Seal the flasks with paraffin after inoculation.

Sterilization: Not specified.

Use: Decomposition of cellulose by mycobacteria. Filter paper showed no signs of being attacked. Growth occurred, however.

Variants: The author gave the following variants:

(a) Used 2.0% peptone and added 1.0% glucose.

(b) Used 2.0% peptone and added 0.4% KNO_3.

Reference: Vierling (1920 p. 206).

628. Matzuschita's Pyrogallic Acid Bouillon

Constituents:

1. Water	1000.0 cc.
2. Meat peptone (Koch)	10.0 g.
3. NaCl	5.0 g.
4. Dextrose (2.0%)	20.0 g.
5. Glycerin (5.0%)	50.0 g.
6. "Eikonogen" (0.1%)	1.0 g.
7. Hydrochinone (0.1%)	1.0 g.
8. Pyrogallic acid (0.1%)	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

(2) Neutralize. Indicator not specified.

Sterilization: Sterilize in the steamer on from 2 to 5 successive days for 15 to 30 minutes. Incubate for 2 days at 37°C. to test sterility.

Use: Cultivation of spore forming bacilli. *Clostridium butyricum, Bacillus oedematis maligni, Bacillus anthracis symptomatica, Bacillus sporogenes, Bocillus botulinus.*

Reference: Matzuschita (1902 p. 287).

629. Bezançon's Glycerol Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose	20.0 g.
3. Glycerol	20.0 g.
4. Peptone	10.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of Hyphomycetes, *Sporotrichum beurmanni.*

Reference: Bezançon (1920 p. 644).

630. Behrens' Tartrate Peptone Solution.

Constituents:

1. Water	1000.0 cc.
2. Starch	10.0 g.
3. Cane sugar	20.0 g.
4. Peptone	5.0 g.
5. Tartaric acid	5.0 g.
6. K_2HPO_4	2.0 g.
7. $MgSO_4$	1.0 g.
8. Copper sulphate 0.0, 0.1, 0.02, 0.1, 0.2, 0.5 or 1.0 g. (per 100.0 cc.)	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Distribute in 100.0 cc. lots.

(3) Prepare a 10.0% solution of copper vitrol.

(4) Add 0.0, 0.1, 0.2, 1.0, 2.0, 5.0 or 10.0 cc. of (3) to each flask.

Sterilization: Method not given.

Use: To show influence of copper on growth of *Oidium fructigenum.* Growth is inhibited by a dilution of about 1.0 g. of copper sulphate per 100.0 cc. (10.0 cc. of a 10.0% solution).

Variants: Author used 50.0 g. sucrose instead of 20.0 g. and omitted the starch.
Reference: Behrens (1898 pp. 775–776).

631. Dombrowski's Glucose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte)	10.0 g.
3. Glucose	50.0 g.
4. K_2HPO_4	2.0 g.
5. $MgSO_4$	1.0 g.
6. NaCl	1.0 g.
7. Lactic acid	

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Acidify slightly with lactic acid.

Sterilization: Not specified.
Use: Cultivation of milk yeasts.
Reference: Dombrowski (1910 p. 358).

632. Zumstein's Citric Acid Peptone Solution (Killer)

Constituents:

1. Water	1000.0 cc.
2. Peptone	5.0 g.
3. Glucose	5.0 g.
4. Citric acid	2.0 g.
5. $MgSO_4 \cdot 7H_2O$	0.2 g.
6. KH_2PO_4	0.5 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.
Sterilization: Not specified.
Use: Cultivation of flagellates, organisms from the soil, etc.
Variants: Dilute the medium 5 to 10 times for cultivation of protozoa.
Reference: Killer (1913 p. 522).

633. Seitz's Double Sugar Peptone Solution (Harvey)

Constituents:

1. Distilled water	1000.0 cc.
2. Lactose	20.0 g.
3. Glucose	0.4 g.
4. Na_2HPO_4	0.5 g.
5. Ammonium sulphate	1.0 g.
6. Sodium citrate	2.0 g.
7. NaCl	5.0 g.
8. Peptone	0.05 g.
9. Azolitmin	0.05 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1. (Method not given.)
Sterilization: Not specified.
Use: Substitute for litmus milk.
Variants: Klimmer specified the use of Witte's peptone and used 0.25 g. Kahlbaum's azolitmin or added 1 or 2 drops of a saturated watery solution of China blue. He used the medium as a whey substitute.
References: Harvey (1921–22 p. 109), Klimmer (1923 p. 208).

634. Müller, Thurgau and Osterwälder's Malic Acid Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte)	10.0 g.
3. H_2KPO_4	1.0 g.
4. $MgSO_4$	0.2 g.
5. $CaCl_2$	0.2 g.
6. Malic acid	1.0 g.
7. Levulose	20.0 g.

Preparation:
(1) Boil 10.0 liters of tap water. (Given as ten liters and not one.)
(2) Filter.
(3) Dissolve 2, 3, 4, 5, 6 and 7 in (2).
(4) Tube.

Sterilization: Sterilize the fractional method.
Use: Cultivation of *Bacterium mannitopoeum* and bacteria from wine and fruit wine. Authors reported that when potassium malate is present a larger ratio of the acid produced is lactic (non volatile) rather than acetic.
Variants: Authors added 1.0% potassium malate.
Reference: Müller, Thurgau and Osterwälder (1912–13 p. 161).

635. Ayers, Rupp and Johnson's Glucose Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Pepsin (Park Davis & Co.)	5.0 g.
3. Peptone (Park Davis & Co.)	5.0 g.
4. K_2HPO_4	3.0 g.
5. NaCl	5.0 g.
6. Glucose	2.5 g.
7. Soap (castor oil)	

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Adjust to pH = 7.6.
(3) Add sufficient quantity of castor oil soap (sodium ricinolate) to lower the surface tension 50 and 43 dynes.

Sterilization: Method not specified.

Use: To study effect of surface tension on growth of streptococci. Author reported that in general when the surface tension was reduced to from 40 to 41 dynes the growth of streptococci was prevented.

Reference: Ayers, Rupp and Johnson (1923 p. 204).

636. Marmier's Glycerol Protease Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	40.0 g.
3. NaCl	15.0 g.
4. Sodium phosphate	0.5 g.
5. Potassium phosphate	0.2 g.
6. Glycerol	40.0 g.

Preparation:

(1) Dissolve the peptone in a certain amount of water.

(2) Add enough $(NH_4)_2SO_4$ to (1) so that the solution is saturated at 100°C.

(3) Boil and then filter.

(4) Dialyse in a current of water for 40 hours.

(5) The liquid may then be reduced to 3.0% peptone and sterilized at 115° if not ready for use.

(6) Instead of dialysing the liquid free from $(NH_4)_2SO_4$ as in step (4), barium hydrate may be added in such an amount so that all the sulphate be precipitated. Filter and keep the filtrate at a temperature near boiling and pass a current of air thru it to free it of ammonia. When free of ammonia pass a current of CO_2 thru the filtrate to remove the excess of barium. Filter.

(7) Make up the ammonia free filtrate (5) or (6) up to 1 liter and dissolve 3, 4, 5 and 6 in it.

(8) Filter and distribute the filtrate in 250.0 cc. Pasteur flasks.

Sterilization: Sterilize at 110°C. (time not specified).

Use: Anthrax toxin production. Marmier reported this medium as being non-toxic for animals.

Reference: Marmier (1895 p. 536).

637. Franklin and Fox's Glycerol Peptone Solution (Owen)

Constituents:

1. Water	5000.0 cc.
2. Glycerol	60.0 g.
3. Peptone	2.0 g.
4. Calcium carbonate (precipitated)	30.0 g.
5. Potassium phosphate	0.5 g.
6. $MgSO_4$	1.0 g.
7. $CaCl_2$	0.5 g.

Preparation:

(1) Dissolve 5, 6 and 7 in 1.

(2) Dissolve 2, 3 and 4 in 2000.0 cc. of (1).

(3) Divide into liter portions.

Sterilization: Sterilize using the intermittent method.

Use: To determine amount of alcohol formed in the fermentation of glycerin by *Bacillus saccharalis.* Author reported that from a liter culture 50.0 cc. of a 1.707% alcohol solution could be obtained.

Reference: Owen (1916 pp. 239–241).

638. Harvey's Glycerol Peptone Solution

Constituents:

1. Peptone (2.0% soln.)	870.0 cc.
2. Glycerol	100.0 cc.
3. NaOH (normal soln.)	30.0 cc.

Preparation:

(1) Prepare a 2.0% peptone solution.

(2) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: Cultivation of *V. cholerae.*

Reference: Harvey (1921–22 p. 87).

639. Waksman's Basal Glycerol Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol	30.0 g.
3. K_2HPO_4	1.0 g.
4. KCl	0.5 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.01 g.
7. Peptone (Witte's)	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 2.0 g. of one of the added nutrients in 1.

(2) Tube in 10.0 to 12.0 cc. quantities.

Sterilization: Sterilize at 15 pounds pressure for 15 minutes.

Use: To study metabolism of actinomycetes.

Added nutrients: The author added 2.0 g. of one of the following salts:

$NaNO_3$

$NaNO_2$

$(NH_4)_2SO_4$
$(NH_4)_2CO_3$

Reference: Waksman (1920 p. 3).

640. Capaldi and Proskauer's Mannitol Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Mannitol (0.1%)	1.0 g.
3. Peptone (Witte) (0.5 or 2.0%)	5.0 or 20.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: To study the fermentation of mannitol by the colon-typhoid group.

Variants: The author gave the following variants:

(a) Substituted Carne, Aschman, Chapoteaut, Drüsen, Hemialbumose, somatose or glutin peptone (Paal) for Witte's peptone.

(b) Used 2.0% Witte's peptone and added sufficient litmus to give a color. Mannitol was fermented with strong acid reaction by the typhoid bacilli after 20 hours. The following salts were added in 0.1% strength. Their presence did not inhibit development of the organisms to any extent:

aluminum acetate
potassium antimonate
sodium arsenate
lead acetate
borax
cadmium chloride
chrome alum
iron alum
potassium ferrocyanate
copper acetate
manganese sulphate
ammonium molybdate
nickel sulphate
sodium nitroprusside
palladium chloride
sublimate
silver sulphate
strontium nitrate
thallium
uranium nitrate
bismuth citrate
zinc sulphate
zinc chloride

(c) Harvey added 47.5 cc. litmus solution to a solution containing 2.0% peptone and 0.1% mannitol.

References: Capaldi and Proskauer (1894 p. 464), (1896 p. 472), Harvey (1921–22 p. 111).

641. Omeliansky's Formate Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Calcium formate	20.0 g.
3. Peptone	2.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Flask.

Sterilization: Method not given.

Use: To study decomposition of formic acid by *Bacterium formicicum*. About 3 times more H_2 was formed than CO_2. Inoculate and seal with a rubber stopper fitted with a tube containing a mercury seal. About 250.0 cc. of air was sealed into the flask with the culture.

Reference: Omeliansky (1903–04 p. 186).

642. Voges and Proskauer's Basal Citrate Peptone Solution

Constituents:

1. Distilled water	100.0 cc.
2. Na_2HPO_4	0.37 g.
3. KH_2PO_4	0.14 g.
4. $CaCl_2$	0.04 g.
5. KCl	0.3 g.
6. Magnesium citrate	0.01 g.
7. Peptone (1.0%)	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 1.0% of one of the added nutrients.

(2) Neutralize with soda.

Sterilization: Not given.

Use: Study fermentation of hemorrhagic septicemia bacteria. Growth reported as being more luxuriant than in bouillon.

Added nutrients: The authors added 1.0% of one of the following:

glucose
mannose
levulose
sucrose
lactose
maltose
raffinose
dextrin
starch (potato)
glycerol
adonitol
dulcitol
mannitol

Variants: The authors used the basic solution without any additions.

Reference: Voges and Proskauer (1898 pp. 22, 28).

643. Behren's Basal Citrate Acid Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	2.0 g.
3. $MgSO_4$	1.0 g.
4. Citric acid	1.0 g.
5. Peptone	20.0 g.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Distribute in 200.0 cc. lots in flasks.
(3) Add one of the combinations given under added nutrients to each flask.

Sterilization: Method not given.

Use: Cultivation of botrytis spores.

Added nutrients: The author added one of the following to 200.0 cc. of the basic solution:

sucrose	0.5 g.
salicin	4.0 g.
salicin	1.0 g.
+ glycerol	1.0 g.
glycerol	1.0 g.

Reference: Behrens (1898 p. 578).

644. V. Freudenreich and Jensen's Lactate Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte)	20.0 g.
3. K_2HPO_4	2.0 g.
4. NaCl	5.0 g.
5. Calcium lactate	20.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Enrichment of propionic acid formers from cheese. Medium is inoculated with a cheese emulsion.

References: V. Freudenreich and Jensen (1907 p. 529), Boekhout and Ott de Vries (1918 p. 130).

645. Baginsky's Lactate Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Sodium lactate	10.0 g.
3. Peptone	2.5 g.
4. K_2HPO_4	0.1 g.
5. $CaCl_2$	0.02 g.
6. $MgSO_4$	0.02 g.
7. $CaCO_3$	0.02 g.

Preparation:
(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Distribute in sterile flasks.

Sterilization: Sterilize in flowing steam on 3 consecutive days for $\frac{1}{2}$ to 1 hour.

Use: To study fermentation by *B. lactis*.

Reference: Baginsky (1888 pp. 434–462).

646. Harvey's Tartrate Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl	5.0 g.
3. K_2HPO_4	3.0 g.
4. Peptone	30.0 g.
5. Ferric tartrate (2.0% soln.)	10.0 cc.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Tube in 10.0 cc. quantities.
(3) Add 0.1 cc. of a 2.0% neutral ferric tartrate solution. A yellowish precipitate forms.

Sterilization: Method not given.

Use: General culture medium.

Reference: Harvey (1921–22 p. 107).

647. Harrison and Vanderleck's Aesculin Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Witte)	20.0 g.
3. Aesculin (Merck)	0.5 g.
4. Iron citrate scales (Merck)	0.5 g.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Adjust the reaction to +0.6.
(3) Tube in 10.0 cc. lots.

Sterilization: Not specified.

Use: Detection of *B. coli* and *B. typhosus*. *B. coli* gives blackened medium. Medium without bile salts blackened quicker than with the bile salt.

Variants: The authors give the following variants:
(a) Addition of 3.0 g. sodium taurocholate.
(b) Use of 10.0 g. peptone instead of 20.0 g. Witte's peptone, 2.5 g. iron citrate scales (Merck) instead of 0.5 g., and 1.0 g. of aesculin instead of 0.5 g.
(c) Same as (b) but add 2.5 g. bile salt (sodium taurocholate).

Reference: Harrison and Vanderleck (1909 pp. 616, 622).

SUBGROUP I-C. SECTION 10

Liquid media or basal solutions containing peptone or similar commercial digests and additional nitrogen compounds of known chemical composition; organic carbon compounds added.

A_1. Organic nitrogen present only as peptone.
B_1. Inorganic nitrogen supplied as ammonium salt.
C_1. Ammonium chloride added.
Jones' Glucose Peptone Solution.... 648
Lustig's Ammonium Chloride Peptone Solution.... 649
Cathelineau's Basal Ammonium Sulphate Peptone Solution.... 650
C_2. Ammonium sulphate added.
Grimbert's Basal Peptone Salt Solution.... 651
Beijerinck's Malate Peptone Solution.... 652
Klecki's Lactose Peptone Solution... 653
Seitz's Lactose Peptone Solution.... 654
C_3. Ammonium salts of phosphoric acid added.
Hauman's Pectin Peptone Solution.. 655
Charpentier's Sucrose Peptone Solution.... 656
B_2. Inorganic nitrogen supplied as nitrate.
C_1. Additional organic carbon supplied as carbohydrates.
D_1. Monosaccharides added.
Lustig's Nitrate Peptone Solution.. 657
Stoklasa's Nitrate Peptone Solution. 658
Stutzer's Nitrate Glucose Solution.. 659
Duchacek's Nitrate Peptone Solution.... 660
D_2. Polysaccharides added.
Khouvine's Cellulose Nitrate Solution.... 661
Omeliansky's Cellulose Peptone Solution (Khouvine).... 662
C_2. Additional Organic carbon supplied as alcohols or organic acids.
Maassen's Nitrate Peptone Solution. 663
Wassermann's Nitrate Peptone Solution.... 664
Duchacek's Nitrate Tartaric Acid Solution.... 665
A_2. Organic nitrogen present in addition to peptone.
B_1. Amino acids added.
C_1. Amino nitrogen supplied as asparagin.
Hiss' Basal Asparagin Peptone Solution.... 666
Henneberg's Basal Asparagin Peptone Solution.... 667
Nicole and Refik-Bey's Asparagin Peptone Solution.... 668
Thoinnessen's Asparagin Peptone Solution.... 669
Bokorny's Asparagin Peptone Solution.... 670
Reichenbach's Lactose Peptone Solution (Quantz).... 671
Frankel's Asparagin Peptone Solution (Tanner).... 672
C_2. Amino nitrogen supplied as tyrosine.
Berthelot's Tyrosine Peptone Solution.... 673
C_3. Amino nitrogen supplied as tryptophane.
Tryptophane Broth (A. P. H. A.).... 674
Frieber's Tryptophane Peptone Solution.... 675
Harvey's Tryptophane Peptone Solution.... 676
B_2. Organic nitrogen other than amino acids added.
C_1. Containing urea.
Hiss' Basal Urea Peptone Solution.. 677
Killer's Urea Peptone Solution...... 678
Cunningham's Urea Peptone Solution.... 679
C_2. Containing hippuric acid.
Ayers and Rupp's Hippurate Peptone Solution.... 680
Ayers and Rupp's Pepsin Peptone Solution.... 681
C_3. Containing Bile Salts.
MacConkey's Bile Salt Peptone Solution.... 682
Harrison and van der Leck's Bile Salt Peptone Solution.... 683

648. Jones' Glucose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. NH_4Cl	5.0 g.
3. Glucose	5.0 g.
4. Peptone	10.0 g.
5. Calcium lactate 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, or 100.0 g.	

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Method not given.

Use: To study fermentation by *B. proteus*. The author reported the more lactate salt present, the less acid and gas were formed.

Variants: The author used sodium or ammonium lactate instead of calcium lactate.

Reference: Jones (1916 p. 40).

649. Lustig's Ammonium Chloride Peptone Solution

Constituents:

1. Water	4000.0 cc.
2. Potassium phosphate	1.0 g.
3. $MgSO_4$ (crystals)	0.2 g.
4. $CaCl_2$ (fusum)	0.1 g.
5. Invert sugar	5.0 g.
6. NH_4Cl	5.0 g.
7. Peptone	1.0 g.
8. $CaCO_3$	15.0 g.

Preparation:

(1) Prepare a salt solution by dissolving 1.0 g. potassium phosphate, 0.2 g. $MgSO_4$ (crystals) and 0.1 g. $CaCl_2$ (fusum), in 1000.0 cc. water.

(2) Prepare an invert sugar solution by dissolving 5.0 g. cane sugar in 1000.0 cc. water and inverting.

(3) Prepare an ammonium chloride solution by dissolving 5.0 g. NH_4Cl in 500.0 cc. water.

(4) Mix 400.0 cc. (1), 240.0 cc. of (2) and 200.0 cc. of (3).

(5) Add and dissolve in (4), 1.0 g. peptone and 15.0 g. of $CaCO_3$ (puriss).

(6) Dilute (5) to 4000.0 cc. with water.

(7) Distribute into well sterilized 100.0 cc. flasks in 30 to 50.0 cc. quantities.

Sterilization: Sterilize well on each of two successive days for an hour.

Use: To study the oxidation of ammonia by a red pigment producing organism isolated from water, and by bacteria from the soil.

References: Lustig (1890 p. 38), Frankland and Frankland (1889 p. 376).

650. Cathelineau's Basal Ammonium Sulphate Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Sodium phosphate	
3. Sodium potassium tartrate	
4. $(NH_4)_2SO_4$	5.0 g.
5. Peptone	10.0 g.
6. $CaCO_3$	

Preparation:

(1) Dissolve 5.0 g. of a mixture of sodium phosphate and sodium potassium tartrate (Seignette's salt) in 1.

(2) Dissolve 5.0 g. $(NH_4)_2SO_4$, 10.0 g. of peptone and one of the added nutrients in (1).

(3) Add an excess of $CaCO_3$.

Sterilization: Not specified.

Use: Cultivation of *Bacillus viridis* (Lesage).

Added nutrients: The author added 2.0% of one of the following materials:

glucose
levulose
lactose
galactose
maltose
sucrose
mannitol
glycerol
succinic acid
tartaric acid
lactic acid
citric acid
butyric acid
glycocolic acid
malic acid
aspartic acid

Reference: Cathelineau (1896 p. 229).

651. Grimbert's Basal Peptone Salt Solution

Constituents:

1. Water	1000.0 cc.
2. Ammonium phosphate	0.4 g.
3. $MgSO_4$	0.4 g.
4. Potassium phosphate	0.2 g.
5. $(NH_4)_2SO_4$	0.2 g.
6. KNO_3	0.2 g.
7. Peptone (dry)	2.5 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 3.0 to 5.0% of one of the added nutrients in 1.

(2) Tube or flask.

Sterilization: Sterilize at 120°C. for 15 to 45 minutes depending on the size of the flask.

Use: To study the fermentation ability of *Bacillus orthobutylicus.*

Added nutrients: The author added 3.0 to 5.0% of any fermentable material.

Reference: Grimbert (1893 p. 359).

652. Beijerinck's Malate Peptone Solution

Constituents:

1. Water (ditch)	1000.0 cc.
2. Potassium malate	2.5 g.
3. Peptone (siccum)	2.5 g.
4. Mohr salt $(FeSO_4(NH_4)_2SO_4 \cdot 6H_2O)$	1.0 g.

Preparation:

(1) Dissolve 2, 3, and 4 in ditch water ("Grabenwasser").

(2) Make alkaline with Na_2CO_3.

Sterilization: Not specified.

Use: To study reduction of sulphates by *Spirillum tenue.*

Reference: Beijerinck (1895 p. 107).

653. Klecki's Lactose Peptone Solution

Constituents:

1. Water	8000 to 10,000.0 cc.
2. Calcium lactate	225.0 g.
3. Ammonium phosphate	0.75 g.
4. Potassium phosphate	0.4 g.
5. $MgSO_4$	0.4 g.
6. $(NH_4)_2SO_4$	0.2 g.
7. Peptone	200.0 g.
8. Lactose	500.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

(2) Add some chalk to each flask.

Sterilization: Method not given.

Use: To study fermentations in cheese. The medium was inoculated with a piece of "Quargel" cheese. *Vibrio butyrique, Bacillus saccharobutyricus,* and other organisms developed. Medium was used under anaerobic as well as aerobic conditions.

Reference: Klecki (1896 p. 173).

654. Seitz's Lactose Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Lactose	20.0 g.
3. Glucose	0.4 g.
4. Na_2HPO_4	1.0 g.
5. Sodium citrate (tribasic)	2.0 g.
6. NaCl	5.0 g.
7. Peptone (Witte sicc)	0.05 g.
8. Azolitmin (Kahlbaum's)	0.25 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

(2) The reaction should be neutral.

(3) The medium is bluish violet in color.

Sterilization: Do not sterilize longer than 30 minutes at 100°C.

Use: Differential medium for colon-typhoid group. Author reported that the colon group produced acid after 5 hours. Typhoid bacilli produced a little acid after 24 hours by dextrose decomposition. Dysentery and paratyphoid organisms acted as typhoid. *Bacillus faecalis alcaligenes* produced an alkaline reaction.

Reference: Seitz (1912 p. 434).

655. Hauman's Pectin Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Pectin	10.0 g.
3. Peptone	1.0 g.
4. Ammonium phosphate	1.0 g.
5. K_2SO_4	0.5 g.
6. $MgSO_4$	0.2 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of *B. coli, B. fluorescens, Cladosporium herbarum, Aspergillus niger, Penicillium glaucum.*

Reference: Hauman (1902 p. 384).

656. Charpentier's Sucrose Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Malto)	10.0 g.
3. Ammonium phosphate (neutral)	0.25 g.
4. Sucrose	100.0 g.
5. Arsenic	Varying amounts

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Adjustment of reaction not given.

(3) To sterile (2) add varying amounts of a sterile solution of arsenic salt.

Sterilization: Sterilize (2) at 120°C. Sterilize the arsenic solution by filtering thru a candle.

Use: To show the effect of metallic salts (arsenic) on the growth of brewers' yeast. The author reported no growth when arsenic was present in one part in 1500.

Reference: Charpentier (1915 p. 452).

657. Lustig's Nitrate Peptone Solution

Constituents:

1. Water	4000.0 cc.
2. Potassium phosphate	1.0 g.
3. $MgSO_4$ (crystals)	0.2 g.
4. $CaCl_2$ (fusum)	0.1 g.
5. Invert sugar	5.0 g.
6. $Ca(NO_3)_2$	5.0 g.
7. Peptone	1.0 g.
8. $CaCO_3$ (puriss)	15.0 g.

Preparation:

(1) Prepare a salt solution by dissolving 1.0 g. potassium phosphate, 0.2 g. $MgSO_4$ (crystals) and 0.1 g. $CaCl_2$ (fusum) in 1000.0 cc. water.

(2) Prepare an invert sugar solution by dissolving 5.0 g. cane sugar in 1000.0 cc. water and inverting.

(3) Dissolve 5.0 g. $Ca(NO_3)_2$ in 500.0 cc. water.

(4) Mix 400.0 cc. (1), 240.0 cc. (2) and 240.0 cc. of (3).

(5) Dissolve 1.0 g. peptone and 15.0 g. $CaCO_3$ (puriss), in (4).

(6) Dilute (5) to 4000.0 cc. with water.

(7) Distribute into well sterilized 100.0 cc. flasks in 30–50 cc. lots.

Sterilization: Sterilize well on each of two successive days for an hour.

Use: To study reduction of nitrates by a red pigment producing organism isolated from water, and by soil forms.

Reference: Lustig (1890 p. 38), Frankland (1889 p. 376).

658. Stoklasa's Nitrate Peptone Solution

Constituents:

1. Water	2000.0 cc.
2. Sodium phosphate	0.5 g.
3. K_2SO_4	0.2 g.
4. $MgCl_2$	0.05 g.
5. Glucose	2.5 g.
6. Peptone	0.5 g.
7. $CaCO_3$	7.5 g.

Preparation:

(1) Dissolve 0.5 g. sodium phosphate, 0.2 g. potassium sulphate and 0.05 g. magnesium chloride in 500.0 cc. water.

(2) Dissolve 2.5 g. glucose in 500.0 cc. water.

(3) Mix 200.0 cc. (1) and 120.0 cc. of (2).

(4) Dissolve 0.5 g. peptone, 7.5 g. calcium carbonate and 5.72 g. calcium nitrate in (3).

(5) Dilute to 2 liters.

(6) Distribute in 500.0 cc. lots.

Sterilization: Sterilize in the steamer for 3 hours.

Use: To study nitrate reduction by *Bacillus megatherium,* (*Bacillus Ellenbachii*) or "Alinit." The author reported that the nitrate was reduced about 8 to 10% after 19 days. Using 1.0 g. of peptone + 6.0 g. $Ca(NO_3)_2$ nitrate reduction was less. When $CaCO_3$ was omitted amount of nitrate reduced with 1.0 g. peptone and 6.0 g. $Ca(NO_3)_2$ was about 10.0%.

Variants: Stoklasa gave the following variants:

(a) Omitted the $CaCO_3$.

(b) Used 1.0 g. of peptone, 6.0 g. of $Ca(NO_3)_2$, with or without $CaCO_3$.

Reference: Stoklasa (1898 p. 285).

659. Stutzer's Nitrate Glucose Solution

Constituents:

1. Water
2. Glucose
3. Peptone
4. KNO_3

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Reaction to be slightly alkaline.

(3) Distribute in 10.0 cc. lots.

Sterilization: Method not given.

Use: To study denitrification by *B. agilis, B. nitrovorus, B. Stutzeri, B. Hartlebi. B. Hartlebi* was the only organisms that decomposed the nitrate.

Reference: Stutzer (1901 p. 84).

660. Duchâcek's Nitrate Glucose Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose	10.0 g.
3. $NaNO_3$	4.0 g.
4. Peptone	10.0 g.
5. Sodium phosphate	0.25 g.
6. $CaSO_4$	0.2 g.
7. $CaCl_2$	0.05 g.
8. $MgCl_2$	0.05 g.
9. $FePO_4$	0.05 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

Sterilization: Not specified.

Use: Cultivation of *Bacillus typhi abdominalis* and *Bact. coli commune.*

Reference: Duchâcek (1904 p. 162).

661. Khouvine's Cellulose Nitrate Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	5.0 g.
3. KNO_3	2.5 g.
4. NaCl	1.0 g.
5. Peptone (pancreatic)	1.0 g.
6. Cellulose	

Preparation:

(1) Dissolve 2, 3, 4, 5 in 1.
(2) Reaction is neutral.
(3) Distribute in 5.0 cc. lots in tubes.
(4) Add 1 square centimeter of cellulose in the form of Berzelius filter paper or a little cellulose precipitated after having been dissolved in Schweitzer's reagent to each tube.

Sterilization: Sterilize for 15 minutes at 110°.

Use: Isolation and enrichment of *B. cellulose dissolvens.*

Variants: The author added 2.0 g. glucose.

Reference: Khouvine (1923 p. 713).

662. Omeliansky's Cellulose Peptone Solution (Khouvine)

Constituents:

1. Distilled water	1000.0 cc
2. K_2HPO_4	1.0 g.
3. $MgSO_4$	0.5 g.
4. KNO_3	3.0 g.
5. Peptone (Witte)	1.0 g.
6. NaCl	trace
7. $CaCO_3$	trace
8. Cellulose	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Reaction is neutral.
(3) Distribute in 5.0 cc. lots in tubes.
(4) Add 1 square centimeter of cellulose in the form of Berzelius filter paper or a little cellulose precipitated after having been dissolved in Schweitzer's reagent to each tube.

Sterilization: Sterilize for 15 minutes at 110°.

Use: Isolation and enrichment of *B. cellulose dissolvens.*

Variants: Khouvine added 2.0 g. glucose.

Reference: Khouvine (1923 p. 713).

663. Maassen's Nitrate Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Sodium phosphate (secondary)	0.5 g.
3. NaCl	0.5 g.
4. Soda	0.5 g.
5. $MgSO_4$	1.0 g.
6. Malic acid	7.0 g.
7. Glycerin	20.0 g.
8. Salt peter	2.5 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1. The malic acid is to be neutralized by the addition of pure soda solution.

Sterilization: Not specified.

Use: To study ammonia production and denitrification.

Reference: Maassen (1902 p. 52).

664. Wassermann's Nitrate Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Na_2HPO_4	3.7 g.
3. KH_2PO_4	1.4 g.
4. $CaCl_2$	0.4 g.
5. KCl	3.0 g.
6. Magnesium citrate	0.1 g.
7. Nitrate	
8. Peptone (Witte) (1.0%)	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 8 in 1.
(2) Add nitrate to (1). (Amount or kind not specified.)

Sterilization: Not specified.

Use: Differentiation of hemorrhagic septicemia bacteria on basis of nitrate reduction and indol production.

Variants: The author used 1.0% of Aschmann's, Merck's or Carne, peptone instead of Witte's. Also used 1.0% of Kuhne's drüsenpeptone, albumoses from Witte's peptone or sodium caseinate instead of Witte's peptone.

Reference: Wassermann (1898 p. 28).

665. Duchàcek's Nitrate Tartaric Acid Solution

Constituents:

1. Water	1000.0 cc.
2. Tartaric acid	10.0 g.
3. $NaNO_3$	4.0 g.
4. Peptone	1.0 g.
5. Sodium phosphate	0.25 g.
6. $CaSO_4$	0.20 g.
7. $CaCl_2$	0.5 g.
8. $MgCl_2$	0.5 g.
9. $FePO_4$	0.5 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.
(2) Neutralize (1) by the addition of soda solution.

Sterilization: Not specified.

Use: Cultivation of *Bacillus typhi abdominalis* and *Bact. coli commune.*

Reference: Duchàcek (1904 p. 162).

666. Hiss' Basal Asparagin Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone	10.0 g.
3. Asparagin	5.0 g.
4. 5.0% litmus solution	10.0 cc.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Dissolve 10.0 g. of one of the added nutrients in (1).

Sterilization: Not specified.

Use: To study fermentation of sugars by the dysentery group.

Added nutrients: The author added 10.0 g. of one of the following:

glucose
maltose
mannitol
sucrose
dextrin

Reference: Hiss (1904–05 p. 29).

667. Henneberg's Basal Asparagin Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4 (0.3%)	3.0 g.
3. $MgSO_4$ (0.01%)	0.1 g.
4. Asparagin (0.3%)	3.0 g.
5. Peptone (1.0%)	10.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 5.0% of one of the added nutrients in 1.

Sterilization: Not specified.

Use: Determine acid production by lactic acid bacteria using various carbon sources. Author reported no growth using starch or dextrin.

Added nutrients: The author added 5.0% of a large variety of carbon sources.

Reference: Henneberg (1903 p. 7).

668. Nicole and Rêfik-Bey's Asparagin Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	20.0 g.
3. NaCl	5.0 g.
4. K_2HPO_4	2.0 g.
5. Asparagin	2.0 g.
6. Ammonium lactate	3.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of cocco bacillus causing goat pneumonia, and growth of the diphtheria bacilli with toxin production.

Reference: Nicole and Rêfik-Bey (1896 p. 325).

669. Thönnessen's Asparagin Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Asparagin	2.0 g.
3. Peptone (siccum)	5.0 g.
4. K_2HPO_4	2.0 g.
5. NaCl	2.0 g.
6. Sodium bicarbonate	1.0 g.
7. $MgSO_4$	0.1 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1. (The commercial K_2HPO_4 must be neutralized with KOH.)

(2) Add 0.1 g. $MgSO_4$ to sterile (1).

Sterilization: Method not given.

Use: Cultivation of anthrax bacilli.

Variants: The author added bouillon. This did not increase the growth of the anthrax bacillus.

Reference: Thönnessen (1902 p. 824).

670. Bokorny's Asparagin Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Asparagin (0.1%)	1.0 g.
3. Peptone (0.025%)	0.25 g.
4. $MgSO_4$ (0.03%)	0.3 g.
5. $CaCl_2$	trace
6. Sucrose (10.0%)	100.0 g.
7. KH_2PO_4 (0.1, 0.2, 0.5, 1.0, 2.0 or 4.0%)	(1.0, 2.0, 5.0, 10.0, 20.0 or 40.0 g.)

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: To study effect of metallic salts on yeast. The author reported that the presence of 4.0% K_2HPO_4 did not produce an inhibitory effect on the growth of yeast. Na_2HPO_4 did not inhibit the development of the yeast.

Variants: The author used 0.5, 1.0, 2.0 or 5.0% Na_2HPO_4 instead of KH_2PO_4.

Reference: Bokorny (1912 p. 119).

671. Reichenbach's Lactose Peptone Solution (Quantz)

Constituents:

1. Water	1000.0 cc.

2. Na_2HPO_4	2.5 g.
3. Asparagin	4.0 g.
4. NaCl	5.0 g.
5. Peptone (Witte)	2.5 g.
6. Lactose	5.0 g.
7. Azolitmin	0.25 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) The reaction is neutral.

Sterilization: Not specified.

Use: Water analysis. *B. coli* grew rapidly in this medium, producing the maximum amount of acid in 4 days.

Reference: Quantz (1914 p. 202).

672. Fränkel's Asparagin Peptone Solution (Tanner)

Constituents:

1. Distilled water	1000.0 cc.
2. $CaH_4(PO_4)_2 \cdot H_2O$	2.0 g.
3. Ammonium lactate	6.0 g.
4. Asparagin	4.0 g.
5. NaCl	5.0 g.
6. N/1 NaOH	20.0 cc.
7. Peptone (4.0%)	40.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Tube.

Sterilization: Sterilize in Arnold.

Use: To study sulphur metabolism of fluorescent bacteria, colon typhoid group and others.

Reference: Tanner (1917 p. 586).

673. Berthelot's Tyrosine Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte)	20.0 g.
3. Glucose	20.0 g.
4. Tyrosine	10.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Production of aromatic hydroxy acids and the study of phenol production by *Bacillus phenologenes.*

Variants: The author used the following variants to study phenol production:

(a) 2.0 g. tyrosine in 1000.0 cc. of peptone water.

(b) 2.0 g. tyrosine and 10.0 g. glucose in 1000.0 cc. peptone water.

Reference: Berthelot (1909 p. 87), (1918 p. 30).

674. Tryptophane Broth (A. P. H. A.)

Constituents:

1. Water (Dist.)	1000.0 cc.
2. Tryptophane	0.3 g.
3. K_2HPO_4	5.0 g.
4. Peptone	1.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Detection of indol production. The Committee stated that some American brands of peptone contained certain uniform amounts of tryptophane. If such peptone be used it is unnecessary to add the tryptophane, but use 5.0 g. peptone per liter.

References: Committee A. P. H. A. (1917 p. 107), (1920 p. 107), (1923 p. 107), Rogers, Clark and Lubs (1918 p. 233), Tanner (1919 p. 47), Giltner (1921 p. 384), Levine (1921 p. 109).

675. Frieber's Tryptophane Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl (0.5%)	5.0 g.
3. Potassium phosphate (0.2%)	2.0 g.
4. $MgSO_4$ (0.02%)	0.2 g.
5. Tryptophane (0.03%)	0.3 g.
6. Peptone (Witte) (0.25%)	2.5 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Indol production by *Bact. pyocyaneus, Staphylococcus pyogenes, Bact. pneumoniae, Bac. diphtheriae,* potato bacillus, disciformans and glanders bacillus. Author reported that the organisms listed above gave positive reactions for indol when using the Salkowski (H_2SO_4 and nitrites) test. Ehrlich's test gave negative indol reaction for these organisms.

Reference: Frieber (1921–22 p. 264).

676. Harvey's Tryptophane Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium lactate	5.0 g.
3. Na_2SO_4	2.0 g.
4. $MgSO_4$	0.2 g.
5. Peptone	30.0 g.
6. Tryptophane 1:1000 soln. 0.1, 0.3 or 0.5 cc.	

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Detection of indol production.

Reference: Harvey (1921–22 p. 102).

677. Hiss' Basal Urea Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Urea	5.0 g.
3. Peptone	10.0 g.
4. 5.0% litmus solution	10.0 cc.

Preparation: (1) Dissolve 2, 3, 4 and 10.0 g. of one of the added nutrients in 1.

Sterilization: Not specified.

Use: To study fermentation of sugars by the dysentery group.

Added nutrients: Hiss added 10.0 g. of one of the following:

glucose
maltose
mannitol
sucrose
dextrin

Reference: Hiss (1904–05 p. 30).

678. Killer's Urea Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. Urea	100.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: Study of decomposition of urea by soil forms and protozoa; production of uric acid and study of nitrogen metabolism.

Variants:

(a) Killer diluted the medium 5 to 10 times for the cultivation of protozoa.

(b) Sears used 20.0 g. peptone and 10.0 g. urea to study uric acid formation and nitrogen metabolism.

References: Killer (1913 p. 523), Sears (1916 p. 132).

679. Cunningham's Urea Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (1.0%)	10.0 g.
3. K_2HPO_4 (0.05%)	0.5 g.
4. Urea (1.0%)	10.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1 by steaming.
(2) Filter.
(3) Dissolve 1.0% urea in 1.
(4) Distribute in 50.0 cc. quantities in 300.0 cc. flasks.

Sterilization: Sterilize intermittently.

Use: Urea decomposition by *Bac. Pasteuri*, *M. ureae liquefaciens* and other soil forms.

Variants: The author added 1.0% glucose.

Reference: Cunningham (1924 p. 145).

680. Ayers and Rupp's Hippurate Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Parke-Davis)	10.0 g.
3. K_2HPO_4	1.5 g.
4. Sodium hippurate	10.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Adjust reaction to pH = 7.2.

Sterilization: Method not given.

Use: To show hydrolysis of sodium hippurate by streptococci.

Reference: Ayers and Rupp (1922. p. 391).

681. Ayers and Rupp's Pepsin Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Park Davis)	10.0 g.
3. Pepsin	5.0 g.
$CaCl_2$	0.03 g.
5. $FeCl_3$ (1.0% soln.)	1 drop
6. Sodium hippurate	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Add NaOH to give pH = 7.1.

Sterilization: Method not given.

Use: To show hydrolysis of sodium hippurate by hemolytic streptococci. Author reported that streptococci from human origin did not show a great increase in volatile acids with sodium hippurate. Udder types showed increase.

Variants: The authors omitted the $FeCl_3$ and added 1.0 g. NaCl. They adjusted the pH from 8.0 to 9.0 and studied the effect of alkaline reaction upon hydrolysis of sodium hippurate by hemolytic streptococci. They reported that if medium was suitable for the growth of streptococci the composition or reaction had no effect on the hydrolysis of sodium hippurate.

Reference: Ayers and Rupp (1922 pp. 390, 393).

682. MacConkey's Bile Salt Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Sodium taurocholate (0.5%)	5.0 g.
3. Glucose (0.5%)	5.0 g.
4. Peptone (2.0%)	20.0 g.
5. Litmus	

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add a sufficient quantity of litmus to give a distinct color.

(3) Tube, and place a Dunham's fermentation tube in each tube.

(4) Adjustment of reaction not given.

Sterilization: Method not given.

Use: Enrichment of colon-typhoid group in water and sewage analysis. The author reported that in this medium *B. coli* and the Gärtner group gave acid and gas in 24 hours.

Variants:

(a) MacConkey (1905) specified the use of distilled water, Witte's peptone, used 5.0 g. glucose or 10.0 g. of any other carbohydrate and used 5.0 cc. of a 1.0% neutral red solution instead of litmus.

(b) MacConkey (1905) specified the use of distilled water, Witte's peptone, used 5.0 cc. of a 1.0% neutral red solution instead of litmus, added 5.0 g. KI and added 10.0 g. mannitol, 5.0 g. glucose or 5.0 g. lactose.

(c) MacConkey (1908) specified the use of distilled water, Witte's peptone, used 2.5 cc. of a 1.0% neutral red solution instead of litmus, added 0.3 g. $CaCl_2$ and added one of the following:

glucose	5.0 g.
lactose	10.0 g.
sucrose	10.0 g.
dulcitol	5.0 g.
adonitol	5.0 g.
inulin	5.0 g.

If tap water be used in the above solution omit the $CaCl_2$.

(d) Roddy (1917) used 2.5 cc. of a 1.0% neutral red solution and added 0.5% glucose, dulcitol or adonitol or 1.0% of any other sugar.

(e) Levine (1921) used 5.0 g. lactose instead of 5.0 g. glucose.

(f) Levine (1921) omitted the litmus, used 1.0% peptone, used ox bile or a 1.0% solution of dry ox bile instead of sodium taurocholate and used 1.0% lactose instead of 0.5% glucose.

(g) Giltner (1921) specified distilled water, used 10.0 g. of lactose instead of 5.0 g. glucose and added 20.0 cc. of 2.0% azolitmin solution instead of litmus.

(h) Harvey (1921–22) used 5.0 cc. of a 1.0% neutral red solution instead of litmus and added 50.0 cc. of a 20.0% solution of any sugar instead of glucose.

(i) Harvey (1921–22) specified distilled water, used 4.0% peptone, 1.0% sodium taurocholate and 1.0% glucose instead of amounts given by MacConkey.

(j) Harvey (1921–22) used 1.0% peptone, 2.5% sodium taurocholate, omitted the litmus and used 1.0% glycerol instead of 0.5% glucose.

(k) Cunningham (1924) specified the use of tap water, used 1.0% lactose instead of 0.5% glucose and used 1.0% Andrades indicator instead of litmus.

References: MacConkey (1901 p. 740), (1905 pp. 334, 337), (1908 p. 325), Abel (1912 p. 226), Roddy (1917 p. 42), Tanner (1919 p. 46), Percival (1920 p. 307), Levine (1921 p. 110), Giltner (1921 p. 382), Harvey (1921–22 pp. 89, 90, 109), Klimmer (1923 p. 214), Cunningham (1924 p. 103).

683. Harrison and van der Leck's Bile Salt Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte) (1.0% or 2.0%)	10.0 or 20.0 g.
3. Sodium taurocholate (Commercial 0.5%)	5.0 g.
4. Aesculin (0.1%)	1.0 g.
5. Iron citrate (0.05%)	0.5 g.

Preparation:

(1) Steam 2, 3, 4 and 5 in 1 for 30 minutes.

(2) Filter.

(3) Adjustment of reaction not given.

(4) Tube.

Sterilization: Sterilize on 3 successive days in the steamer.

Use: Presumptive test for colon group in water analysis. Colon bacilli blacken the medium.

Variants:

- (a) Authors used 0.05% aesculin, and 0.3% sodium taurocholate instead of amount given above.
- (b) Authors used 0.25% iron citrate, and 0.25% sodium taurocholate.
- (c) Giltner used 0.1 g. aesculin instead of 1.0 g. aesculin.

References: Harrison and van der Leck (1908 p. 312), (1909 pp. 549, 616, 622), Giltner (1921 p. 382).

SUBGROUP I-C. SECTION 11

Liquid media, or basal solutions, containing peptone (or other commercial digest) and in addition, one other organic constituent of unknown chemical composition, exclusively of plant or soil origin.

A_1. Yeast or yeast derivatives added.

Sherman's Basal Yeast Peptone Solution.......... 684
Cannon's Basal Yeast Peptone Solution.......... 685
Ayers and Mudge's Basal Yeast Peptone Solution.......... 686
Ayers' Basal Cerevisine Peptone Solution.......... 687
Ayers and Rupp's Yeast Infusion Peptone Solution.......... 688
Ayers and Rupp's Acid Yeast Infusion Peptone Solution.......... 689
Ayers and Mudge's Yeast Infusion Peptone Solution I.......... 690
Ayers and Mudge's Autolized Yeast Peptone Solutions.......... 691
Spronck's Yeast Infusion Peptone Solution.......... 692
Cohen and Clark's Yeast Infusion Peptone Solution.......... 693
Ickert's Yeast Extract Peptone Solution.......... 694
Ickert's Yeast Infusion Peptone Solution.......... 695
Fred and Peterson's Yeast Infusion Peptone Solution.......... 696
Ventre's Basal Yeast Ash Peptone Solution.......... 697
Henneberg's Glucose Yeast Infusion Solution.......... 698
Dietrich's Glycerol Yeast Infusion Peptone Solution.......... 699

A_2. Bacterial Derivatives Added.

Thjötta's Basal Bacterial Infusion Peptone Solution.......... 700

A_3. Fungus derivatives added.

Wiegert's Fungus Infusion Peptone Solution.......... 701

A_4. Derivatives of other plants added.

B_1. Worts, malt or other derivatives added.

Will and Wanderscheck's Sulphur Wort Solution.......... 702
Gottheil's Mannitol Wort Solution.......... 703
de Kruyff's Malt Extract Peptone Solution.......... 704
Zikes' Wort Peptone Solution.......... 705
Peklo's Wort Peptone Solution.......... 706

B_2. Materials other than worts or malts added.

Otabe's Wheat Infusion Peptone Solution.......... 707
Ayers and Mudge's Cabbage Infusion Peptone Solution.......... 708
Kaufmann's Jequirity Seed Infusion Peptone Solution.......... 709
Zikes' Must Peptone Solution.......... 710
Henneberg's Prune Infusion Peptone Solution.......... 711
Miquel's Wood Ash Peptone Solution (Besson).......... 712

684. Sherman's Basal Yeast Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	20.0 g.
3. Dried yeast	10.0 g.
4. Na_2HPO_4	5.0 g.

Preparation: (1) Dissolve 2, 3, 4 and the sodium salt of one of the acids given under added nutrients in 1.

Sterilization: Not specified.

Use: To study the production of CO_2 from organic salts by the kefir type of streptococcus. Author reported that none of the organic acid salts aided in CO_2 production.

Added nutrients and variants:

- (a) The author added the sodium salt of one of the following organic acids:
  formic
  acetic
  propionic
  butyric
  caproic
  lactic
  malic
  valeric
  oxalic
  tartaric
  citric or
  succinic acid

(b) The author omitted the Na_2HPO_4 and used 10.0 g. of peptone instead of 20.0 g. and added 10.0 g. of lactic acid in the form of sodium lactate.

Reference: Sherman (1921 p. 129), (1921 p. 383).

685. Cannon's Basal Yeast Peptone Solution

Constituents:

1. Tap water	1000.0 cc.
2. Peptone (Armours)	10.0 g.
3. Yeast (dried autolyzed)	10.0 g.
4. NaCl	5.0 g.

Preparation:

(1) Boil 2, 3 and 4 in 1 for 15 minutes.
(2) Flask and autoclave.
(3) When cold shake well and filter until clear through paper.
(4) Add 1% of the added nutrients.

Sterilization: Sterilize in the Arnold sterilizer for 20 minutes on each of 3 successive days.

Use: Cultural study of aciduric bacteria.

Added nutrients: The author added 1.0% of any carbohydrate. The aciduric bacteria do not develop on the basic medium, but when a suitable carbohydrate is added the growth is luxuriant, producing a pH less than 5.

Reference: Cannon (1924 p. 229).

686. Ayers and Mudge's Basal Yeast Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Difco)	10.0 g.
3. Yeast (Autolized)	10.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Adjust to pH = 7.4.
(3) Distribute in 50.0 cc. quantities and add 0.5 cc. of one of the added nutrients to each flask.

Sterilization: Sterilize at 15 pounds for 30 minutes.

Use: To study growth promoting substances found in oils and fats for streptococci. Authors reported that sesame oil and chaulmoogra oil inhibited growth, while even solid paraffin stimulated growth. Growth stimulation probably not due to vitamin A, for mineral oils do not contain this vitamin.

Added nutrients: The author added 0.5 cc. of one of the following fats or oils to each 50.0 cc. lot of the basic solution:

Sesame
Chaulmoogra
Castor
Corn
Rape seed
Lumbang
Cocoanut
Peanut: refined, crude
Soy bean
Olive
Chia seed
Mustard seed
Okra seed
Linseed
Lard: Open kettle, Steam refined
Butterfat
Cod liver
Mineral 1 and 2%
Vaseline 1 and 2%
Paraffine 1 and 2%

Reference: Ayers and Mudge (1922 p. 458).

687. Ayers' Basal Cerevisine Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Cerevisine (a dry yeast preparation)	10.0 g.
3. Peptone	10.0 g.

Preparation: (1) Dissolve 2, 3 and 10.0 g. of one of the added nutrients in 1.

Sterilization: Not specified.

Use: To study hydrogen ion concentration in culture of streptococci. Author reported two limiting pH zones, pH = 4.6 to 4.8 and pH = 5.5 to 6.0.

Added nutrients: Ayers added 10.0 g. of one of the following materials:

glucose
lactose
sucrose
raffinose
mannitol
inulin

Reference: Ayers (1916 p. 84).

688. Ayers and Rupp's Yeast Infusion Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Dry fresh yeast	10.0 g.
3. Peptone	10.0 g.

Preparation:

(1) Prepare an infusion by mixing 1.0% of dry fresh yeast with cold distilled water. Allow to stand 10 minutes. Steam in Arnold for 3 minutes and filter. (If filtrate not clear add kieselguhr or Merck's dialysed iron and filter again).
(2) Add 1.0% peptone to yeast extract.
(3) Adjust to pH = 7.5, heat and filter.
(4) Tube.

Sterilization: Method not given.

Use: General culture medium. Authors reported good growth of streptococci.

Variants:

(a) Fresh yeast may be autolysed for 24 hours at 45–50°C. and then dried at low temperatures. Then add peptone and adjust to pH = 7.4.

(b) Dry yeast may also be autolysed and then used.

Reference: Ayers and Rupp (1920 p. 90–91).

689. Ayers and Rupp's Acid Yeast Infusion Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Yeast (dry fresh)	10.0 g.
3. Peptone	10.0 g.

Preparation:

(1) Add 10.0 g. dried fresh yeast to 400.0 cc. distilled H_2O and 50 N/1 HCl.
(2) Heat in autoclave at 14 pounds for ½ hour.
(3) After heating add 50.0 cc. N/1 NaOH.
(4) Cool and filter until clear.
(5) Adjust to pH = 7.5.
(6) Make up extract to 1000.0 cc. with distilled water to make a 1.0% solution.
(7) Dissolve the peptone in (6).
(8) Adjust to pH = 7.5.
(9) Heat and filter.
(10) Tube.

Sterilization: Method not given.

Use: General culture medium.

Reference: Ayers and Rupp (1920 p. 95).

690. Ayers and Mudge's Yeast Infusion Peptone Solution I

Constituents:

1. Water	1000.0 cc.
2. Yeast	10.0 g.
3. Peptone (Difco)	10.0 g.

Preparation:

(1) Add 10.0 g. yeast to 200.0 cc. distilled water, allow to stand about 1 hour, shaking before filtering.
(2) Add 50.0 g. Lloyd's Reagent (Fuller's earth) and HCl to make solution 0.01 normal HCl.
(3) Shake every ½ hour for four hours and filter.
(4) Make up filtrate to 1000.0 cc.
(5) Add 1.0% Difco peptone.
(6) Adjust to pH = 7.2.
(7) Steam for 15 minutes.
(8) Filter and flask.

Sterilization: Method not given.

Use: To show influence of water-soluble B vitamin on growth of streptococci. This medium should not contain water-soluble B from yeast. It gave fairly good growth, about same as regular yeast extract medium.

Reference: Ayers and Mudge (1922 p. 453).

691. Ayers and Mudge's Autolyzed Yeast Peptone Solutions

Constituents:

1. Water	1000.0 cc.
2. Yeast (autolized)	10.0 g.
3. Peptone (Difco)	10.0 g.

Preparation:

(1) Add 10.0 g. autolized yeast to 500.0 cc. boiling H_2O containing 0.01% acetic acid.
(2) Stir for some time.
(3) Filter and concentrate filtrate to 300.0 cc. at about 80°C.
(4) To this filtrate add 95.0% alcohol to get concentration of about 52.0%.
(5) Allow to stand over night at about 5°C.
(6) Filter.
(7) Dissolve the precipitate in 1000.0 cc. distilled water.
(8) Dissolve 1.0% peptone in (6).
(9) Adjust to pH = 7.2.
(10) Filter.
(11) Distribute as desired.

Sterilization: Method not given.

Use: To study the effect of vitamins on the growth of streptococci. The medium given above should contain some soluble B vitamin. Fair growth was obtained.

Variants: The author prepared the following yeast infusions and used them instead of the solution obtained from step (7) as above in preparing media.

(a) This medium should contain a considerable quantity of water soluble B vitamin.

(1) Take the filtrate from step (6) above and concentrate to 200.0 cc.
(2) Add sufficient 95.0% alcohol to give a 79.0% alcohol solution.
(3) Allow to stand over night at 5°C.

(4) Filter.
(5) Dissolve the precipitate in 1000.0 cc. distilled water.
(6) Use this solution for preparing the medium instead of (6) above.

(b) This medium should contain little, if any, water soluble B vitamin.
(1) Take the filtrate from variant (a) step (4) and concentrate to 100.0 cc.
(2) Add 95.0% alcohol to get a concentration of 90.0% alcohol.
(3) Allow to stand over night at 5°C.
(4) Filter.
(5) Evaporate the filtrate to dryness.
(6) Dissolve in 1000.0 cc. distilled water.
(7) Use this solution for preparing the medium instead of (6) in the original medium.

(c) This medium should contain water soluble B vitamin.
(1) Extract 10.0 g. autolized yeast in a Soxhlet apparatus for 8 hours with 95.0% alcohol. Fresh alcohol then added and continued another 8 hours. Repeat this action for 40 hours, breaking up the yeast clumps in thimble several times.
(2) Evaporate alcohol after extraction at 60–80°C.
(3) Add residue to 500.0 cc. distilled water.
(4) Add (3) to 500.0 cc. of a 2.0% Difco peptone solution.
(5) Adjust, heat, filter and sterilize as for the original medium.

(d) This medium should contain no water soluble B vitamin.
(1) Dissolve the residue from variant (c) step (1) in 500.0 cc. distilled water.
(2) Add (1) to 500.0 cc. of 2.0% Difco peptone solution and prepare the medium as in variant (c) step (4) and (5).

(e) This medium should contain water soluble B vitamin.
(1) Add 10.0 g. autolized yeast to 500.0 cc. of distilled water.
(2) Add (2) to 500.0 cc. of a 2.0% Difco peptone solution prepare the medium as in variant C step (4) and (5).

Reference: Ayers and Mudge (1922 pp. 450, 451).

692. Spronck's Yeast Infusion Peptone Solution

Constituents:

1. Water	5000.0 cc.
2. Yeast	1000.0 g.
3. NaCl	25.0 g.
4. Peptone (Witte)	100.0 g.

Preparation:
(1) Boil 1 kilogram of commercial yeast in 5 liters of water for 20 minutes, stirring constantly.
(2) Pour into glass cylinders and allow to stand for 24 hours.
(3) Decant the clear fluid.
(4) Carefully acidulate the fluid and add 5.0 g. NaCl and 20.0 g. Witte's peptone per liter of fluid.
(5) Neutralize by the addition of soda and then add 7.0 cc. of a normal soda solution for each liter of medium.
(6) Heat, and filter on a paper.
(7) Distribute in flasks.

Sterilization: Sterilize at 120°C. (time not specified).

Use: Production of diphtheria toxin.

References: Spronck (1898 p. 702), Tanner (1919 p. 57), Besson (1920 p. 35), Harvey (1921–22 p. 120).

693. Cohen and Clark's Yeast Infusion Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Brewer's yeast	10.0 g.
3. Peptone (Difco)	10.0 g.
4. K_2HPO_4	5.0 g.
5. Succinic acid	2.0 g.
6. Glucose	10.0 g.

Preparation:
(1) Extract 10.0 g. Brewer's yeast in 1000.0 cc. of water containing Difco peptone 1.0%, K_2HPO_4 0.5%, succinic acid 0.2% and glucose 1.0% (method not given).
(2) Filter.
(3) Adjust to desired pH with NaOH or HCl.

Sterilization: Method not specified.

Use: To study H-ion influence on growth of *B. bulgaricus* and *B. coli.*

Reference: Cohen and Clark (1919 p. 421).

694. Ickert's Yeast Extract Peptone Solution

Constituents:

1. Water	1000.0 cc.

2. Yeast extract	10.0 g.
3. Peptone	10.0 g.

Preparation:

(1) Stir 10.0 g. of yeast extract (commercial product from E. stock) and boil for one hour in a steamer.

(2) Clarify by repeated filtering thru paper, by addition of serum, or egg white, or by the addition of 5.0 cc. liquid Ferric oxychlorate, and then filtering.

(3) Add 1.0% peptone but no NaCl as the extract contains 50.0% NaCl.

Sterilization: Not specified.

Use: Meat infusion substitute for the preparation of special media, Endoagar, lactose litmus agar, etc. Do not heat over 100°C. or a brown color and a precipitate will develop.

Reference: Ickert (1918 p. 186).

695. Ickert's Yeast Infusion Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Pressed yeast	30.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.

Preparation:

(1) Suspend 30.0 g. (40.0 g. when preparing agar medium) of pressed yeast in 1 liter of water.

(2) Allow to infuse for one hour.

(3) Boil for 2 hours in the steamer.

(4) Clarify by the addition of 5.0 cc. of ferric oxychlorate while hot, and filter twice thru a double filter.

(5) Add 0.5% NaCl and 1.0% peptone.

Sterilization: Not specified.

Use: Meat infusion substitute for the preparation of special media, Endo, litmus lactose agar, etc. Do not heat over 100°C. or a brown color and a precipitate will form.

Reference: Ickert (1918 p. 186).

696. Fred and Peterson's Yeast Infusion Peptone Solution

Constituents:

1. Fresh yeast water extract	1000.0 cc.
2. K_2HPO_4	5.0 g.
3. Difco peptone	5.0 g.
4. Xylose	20.0 g.
5. Brom cresol purple	

Preparation:

(1) Preparation or composition of fresh yeast water extract not given.

(2) Dissolve 2, 3 and 4 in 1.

(3) Adjustment of reaction not given.

(4) Flask in 250.0 cc. lots.

(5) When inoculating add a few drops of brom cresol purple.

Sterilization: Sterilize at 15 pounds pressure for 30 minutes in the autoclave.

Use: To determine fermentation of xylose.

Reference: Fred and Peterson (1920 p. 540).

697. Ventre's Basal Yeast Ash Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose	170.0 g.
3. Malt dust extract	2.0 g.
4. Peptone (malto peptone)	0.5 g.
5. Yeast ashes	0.5 g.
6. $(NH_4)_2HPO_4$	1.0 g.
7. K_2CO_3	1.85 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Add one of the added nutrients and 1.85 g. of K_2CO_3 to (1).

Sterilization: Sterilize by heating in the autoclave at 120°C., by filtration thru a candle or by treating with H_2SO_4 (250 mg. H_2SO_4 per liter) (details of method not given).

Use: Cultivation of yeast.

Added nutrients and variants:

(a) The author added one of the following:

tartaric acid	4.0 g.
malic acid	5.0 g.
citric acid	0.5 g.

(b) Used the basic solution without the addition of other materials.

Reference: Ventre (1914 p. 198).

698. Henneberg's Glucose Yeast Infusion Solution

Constituents:

1. Yeast infusion (1.0%)	1000.0 cc.
2. Peptone (1.0%)	10.0 g.
3. Glucose (5.0%)	50.0 g.

Preparation:

(1) Prepare a 1.0% yeast infusion (method not given).

(2) Dissolve 2 and 3 in (1).

Sterilization: Not specified.

Use: Cultivation of lactic acid bacteria.
Reference: Henneberg (1903 p. 8).

699. Dietrich's Glycerol Yeast Infusion Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte) (1.0%)	10.0 g.
3. Yeast (dry 1.0%)	10.0 g.
4. Glycerol 3.0%	30.0 g.

Preparation:
(1) Add 1.0% dry yeast to a 1.0% Witte peptone solution.
(2) Boil 2 or 3 times in the autoclave.
(3) Add powdered egg white or dry serum to clarify.
(4) Add 3.0% glycerol.
(5) If the medium is not clear allow to stand for several days and decant.

Sterilization: Method not given.

Use: Cultivation of tubercle bacilli and preparation of tuberculin.

Reference: Dietrich (1921 p. 406).

700. Thjötta's Basal Bacterial Infusion Peptone Solution

Constituents:

1. Peptone water	1000.0 cc.
2. Bacterial extract	40.0 cc.
3. Andrade indicator	

Preparation:
(1) Exact composition of peptone water not given.
(2) Add andrades indicator (amount not given).
(3) Dissolve 1.0% of one of the added nutrients in (2).
(4) Distribute in 5.0 cc. lots.
(5) Suspend a 24 hour growth of *B. proteus* on agar plates, in normal saline solution using 1.0 cc. for each plate.
(6) Collect in a sterile centrifuge tube.
(7) Boil 5 minutes, centrifuge and pipette off the supernatent fluid. (Reaction pH = 7.6.)
(8) Add 0.2 cc. of (7) to each tube of (4).

Sterilization: Not specified.

Use: To study bacterial nutrition. All sugars supported the growth of *Bacillus influenzae*. Acid was produced (redness of medium) only with glucose. Filtering the extract thru a Berkefeld filter did not change its value for promoting growth.

Added nutrients: The author added 1.0% of one of the following materials:

lactose
raffinose
maltose
glucose
mannitol
inulin
salicin
sucrose

Reference: Thjötta (1921 p. 770).

701. Wiegert's Fungus Infusion Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Fungus	250.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.

Preparation:
(1) Chop 250.0 g. of fungus into small pieces and place in a steamer for several hours.
(2) Evaporate the fluid from the fungus to a thick dark brown extract.
(3) Add 1.0 or 2.0% of (2), 1.0% peptone and 0.5% NaCl to water.

Sterilization: Not specified.

Use: The fungus extract is substituted for meat extract.

Reference: Wiegert (1922–23 p. 110).

702. Will and Wanderscheck's Sulphur Wort Solution

Constituents:

1. Beer Wort	1000.0 cc.
2. Peptone (Witte)	15.0 g.
3. Sulphur	3.0 g.

Preparation: (1) Add 1.5% Witte's peptone and 0.3% sulphur to sterile beer wort contained in Pasteur flasks.

Sterilization: Method not given.

Use: Study of hydrogen sulphide production by yeast. Authors reported that the addition of peptone to beer wort containing sulphur did not increase the H_2S production.

Reference: Will and Wanderscheck (1906 p. 308).

703. Gottheil's Mannitol Wort Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	5.0 g.
3. Beerwort (dry)	15.0 g.
4. Mannitol	10.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: General culture medium for organisms found in the soil, on roots and rhizomes.

Reference: Gottheil (1901 p. 432).

704. de Kruyff's Malt Extract Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Malt extract (Merck)	7.0 g.
3. Maltose	10.0 g.
4. Glucose	10.0 g.
5. Peptone	0.5 g.
6. Asparagin	0.5 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) After sterilization add H_3PO_4 so that 100.0 cc. of the medium will neutralize 3.0 cc. of N/1 NaOH.

Sterilization: Method not given.

Use: Enrichment of yeast—*Saccharomyces javanicus.*

Reference: de Kruyff (1908 p. 617).

705. Zikes' Wort Peptone Solution

Constituents:

1. Wort	1000.0 cc.
2. Glucose (5.0%)	50.0 g.
3. Peptone (1.0%)	10.0 g.

Preparation: (1) Dissolve 5.0% glucose and 1.0% peptone in wort.

Sterilization: Not specified.

Use: Cultivation of *Apiculatus* yeast, *Torula alba, Torula Molischiana, Mycoderma cerevisiae, Blastoderma salmonicolor.*

Reference: Zikes (1911 p. 147).

706. Peklo's Wort Peptone Solution

Constituents:

1. Distilled water	200.0 cc.
2. Beer wort	800.0 cc.
3. K_2HPO_4	15.0 g.
4. K_2CO_3	2.0 g.
5. $MgSO_4$	1.8 g.
6. Peptone (Witte 0.5%)	5.0 g.

Preparation:

(1) Mix 1 and 2.

(2) Dissolve 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of plant actinomyces.

Reference: Peklo (1910 p. 551).

707. Otabe's Wheat Infusion Peptone Solution

Constituents:

1. Distilled water	1600.0 cc.
2. Wheat	1.0 pound
3. Diastase	0.5 g.
4. Peptone	10.0 g.
5. NaCl	5.0 g.

Preparation:

(1) Roast wheat (with or without husks) in an iron pan until it becomes brown.

(2) Put 1 pound of (1) into 1600.0 cc. of distilled water. Do not wash the wheat.

(3) Boil in a Koch boiler for 30 minutes.

(4) Strain thru a clean cloth.

(5) Make up to 1000.0 cc. with distilled water if the volume is below this quantity.

(6) Add 0.5 g. of taka-diastase or ordinary diastase and shake the flask well.

(7) Keep at 30 to 40°C. for 30 minutes.

(8) Filter. The filtrate should be quite transparent with a yellowish color, nearly the same as meat broth. It has a sweet smell and of a slightly alkaline reaction.

(9) Add 5.0 g. NaCl and 10.0 g. peptone albumin.

(10) Boil and filter.

(11) To prepare wheat agar add 15.0 g. (in winter) and 20.0 g. (in summer) of agar to the filtrate of (10). Prepare almost as in the same manner as in the preparation of meat agar (details not given).

Sterilization: Not specified.

Use: Substitute for meat infusion medium. May be used as a basis for special media.

Reference: Otabe (1919 p. 576).

708. Ayers and Mudge's Cabbage Infusion Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (Difco)	20.0 g.
3. Cabbage	100.0 g.

Preparation:

(1) Soak 100.0 g. finely minced cabbage in 300.0 cc. distilled water in ice box for 48 hours.

(2) Steam 30 minutes and filter.
(3) Distribute 2.0% Difco peptone solution into a series of flasks in 50.0 cc. quantities.
(4) Add varying amounts of filtrate of (2)—1.0 cc., 5.0 cc. and 50.0 cc.
(5) Make each flask to 100.0 cc. volume with water.
(6) Adjust to pH = 7.2.
(7) Steam and filter.
(8) Tube.

Sterilization: Method not given.

Use: To study growth promoting substances for streptococci. Author reported that the more cabbage extract added, the better the growth.

Reference: Ayers and Mudge (1922 p. 455).

709. Kaufmann's Jequirity Seed Infusion Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Jequirity seeds	100.0 g.
3. Peptone	20.0 g.
4. NaCl	5.0 g.

Preparation:
(1) Grind 100.0 g. Jequirity seeds in a mortar.
(2) The peeled or shelled seeds now weigh about 80.0 g.
(3) Add to 1000.0 cc. of water.
(4) Boil in a steam sterilizer for about 2 hours.
(5) Cool and filter.
(6) Dissolve peptone in (5).
(7) Add 8 drops of concentrated soda solution to each 100.0 cc. of (6).
(8) Distribute into test tubes.

Sterilization: Sterilize in the usual manner (Method not given).

Use: General culture medium. *Bacillus pyocyaneues* and many other organisms grew very well.

Variants: The author gave the following variants:
(a) Addition of 5.0 g. NaCl.
(b) Addition of 60.0 g. glycerol.

Reference: Kaufmann (1891 p. 68).

710. Zikes' Must Peptone Solution

Constituents:

1. Must	1000.0 cc.
2. Glucose (5.0%)	50.0 g.
3. Peptone (1.0%)	10.0 g.

Preparation: (1) Dissolve 5.0% glucose and 1.0% peptone in must.

Sterilization: Not specified.

Use: Cultivation of yeasts, *Torula alba, Torula Molischiana, Mycoderma cerevisiae, Blastoderma salmonicolor.*

Reference: Zikes (1911 p. 147).

711. Henneberg's Prune Infusion Peptone Solution

Constituents:

1. Prune infusion (1.0%)	1000.0 cc.
2. Peptone (1.0%)	10.0 g.
3. Glucose (5.0%)	50.0 g.

Preparation:
(1) Method of preparation of 1.0% prune infusion not given.
(2) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: Cultivation of lactic acid bacteria.

Reference: Henneberg (1903 p. 8).

712. Miquel's Wood Ash Peptone Solution (Besson)

Constituents:

1. Water	1000.0 cc.
2. NaCl	5.0 g.
3. Peptone (Chapoteaut)	20.0 g.
4. Wood ashes	0.1 g.

Preparation:
(1) Dissolve 2 and 3 in 1 by heating.
(2) Add 0.1 g. of wood ashes to (1).
(3) Boil.
(4) Filter thru paper.
(5) Add tartaric acid until the solution is neutral to litmus.
(6) Boil 5 minutes.
(7) Filter.
(8) Make up to 1000.0 cc.
(9) Tube.

Sterilization: Sterilize at 113°C.

Use: General culture medium.

Reference: Besson (1920 p. 30).

SUBGROUP I-C. SECTION 12

Liquid media containing peptone (or other commercial digest) and at least one other organic nitrogen source of animal origin exclusive of extracts or infusions.

A_1.* Animal cells or tissues employed.
B_1.* Red blood cells used.
Rivers and Kohn's Blood Cell Peptone Solution 713

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*See A_2, A_3, B_2, B_3 and B_4 next page.

Wordley's Blood Cell Peptone Solution.............................. 714

B_2. Brain tissue used.

Hall's Brain Peptone Solution....... 715

B_3. Muscular tissue (skeletal) used.

Hall and Peterson's Meat Mash Peptone Solution.................... 716
Kitasato and Weyl's Chopped Beef Peptone Medium.................. 717

B_4. Other animal tissues used.

Jablon and Pease's Liver Peptone Medium.......................... 718
Deycke's Albumin Peptone Solution. 719
Harvey's Egg Peptone Solution..... 720
Weissenbach's Albumin Peptone Solution.......................... 721
Sherman and Albus' Basal Yeast Fat Peptone Solution.................. 722
Weiss and Wilkes-Weiss' Whole Egg Solution.......................... 723

A_2. Animal fluids employed.

B_1. Whole blood used.

Kelser's Blood Infusion Peptone Solution.......................... 724
Wien's Blood Peptone Solution (Klimmer)....................... 725

B_2. Serum used.

Kligler's Peptone Serum Blood Medium.............................. 726
Hiss' Basal Serum Peptone Solution. 727

B_3. Other serous fluids used.

Emile-Weil's Pleuritic Serum Peptone Solution.................... 728
Hiss' Basal Ascitic Fluid Peptone Solution (Park, Williams and Krumwiede)...................... 729

B_4. Bile used.

Abbott's Bile Peptone Solution..... 730
Stitt's Bile Peptone Solution........ 731
Boudeille's Bile Peptone Solution... 732
Jackson and Melia's Bile Peptone Solution.......................... 733
Olszewski and Köhler's Bile Peptone Solution.......................... 734
Muer and Harris' Brilliant Green Lactose Peptone Solution......... 735
Conradi's Bile Peptone Solution..... 736

A_3. Animal secretions, excretions or their derivatives employed.

B_1. Milk or its derivatives used.

Vierling's Whey Peptone Solution.. 737
Park, Williams and Krumwiede's Whey Peptone Solution........... 738
Cunningham's Peptone Whey Solution.............................. 739
Kan-Ichiro Morishima's Nutrose Peptone Solution.................. 740
Elser and Huntoon's Basal Nutrose Solution.......................... 741
Loeffler's Malachite Green Nutrose Solution.......................... 742
Seliber's Basal Casein Peptone Solution.............................. 743
Albus and Holm's Milk Yeast Medium (Medium Y)................ 744

B_2. Excretions used.

Heller's Urine Peptone Solution.... 745
Piorkowski's Urine Peptone Solution. 746
Khouvin and Delaunay's Cellulose Fecal Infusion Solution........... 747

713. Rivers and Kohn's Blood Cell Peptone Solution

Constituents:

1. Distilled water	....	1000.0 cc.
2. Peptone (Fairchilds)	....	20.0 g.
3. NaCl	....	5.0 g.
4. Red blood cells	....	10.0 cc.

Preparation:

(1) Dissolve by boiling 2 and 3 in 1.
(2) Adjust to pH = 7.4.
(3) Add 10.0 cc. of washed blood cells and heat to 95°C. (Time not given.)
(4) Filter thru paper.
(5) After sterilization tube or flask in sterile containers and incubate to test sterility.

Sterilization: Filter thru a Mandler filter to sterilize.

Use: Cultivation of influenza bacillus causing meningitis.

Variants: Added some KNO_3, amount not given.

Reference: Rivers and Kohn (1921 p. 479).

714. Wordley's Blood Cell Peptone Solution

Constituents:

1. Peptone water	....	100.0 cc.
2. Red blood cells (Human)	....	2.0 cc.

Preparation:

(1) Exact composition of peptone water not given.
(2) Distribute in 5.0 lots.
(3) To each tube of (2) add 0.1 cc. of centrifuged human red cells.
(4) Shake well.

Sterilization: Not specified.

Use: To study haemolysis by streptococci.

Reference: Wordley (1921 p. 66).

715. Hall's Brain Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Sheep brain	1000.0 cc.
3. Peptone	40.0 g.
4. Dextrose	20.0 g.

Preparation:

(1) Boil sheep brains with an equal volume of distilled water.
(2) Decant water (save) and press brains thru a potato ricer.
(3) Add 2.0% peptone and 0.1% glucose to the water decanted from (2).
(4) Mix (3) and the brain tissue after it has been passed thru a potato ricer.
(5) Tube by punching thru the filling funnel with a glass rod, filling tubes about half full.

Sterilization: Sterilize intermittently in the Arnold sterilizer. Five daily runs of 30 minutes each are recommended.

Use: To enrich anaerobes. Growth indicated by turbidity and often gas production.

Reference: Hall (1920 p. 579).

716. Hall and Peterson's Meat Mash Peptone Medium

Constituents:

1. Water	1000.0 cc.
2. Ground meat	1.0 lb.
3. NaCl	5.0 g.
4. Peptone (Bacto)	20.0 g.

Preparation:

(1) Dissolve 3 and 4 in 1.
(2) Add 2 to (1).
(3) Adjust to pH = 7.2 by the addition of NaOH.
(4) Tube.

Sterilization: Sterilize in Arnold (streaming steam) on 3 successive days.

Use: Study of toxin production in vitro. Author reported that the addition of 1.0% glucose did not aid toxin production. The marble seal was used to secure better anaerobic conditions.

Variants: The author added 10.0 g. glucose.

Reference: Hall and Peterson (1923 p. 327).

717. Kitasato and Weyl's Chopped Beef Peptone Medium

Constituents:

1. Water	2500.0 cc.
2. Beef	1250.0 g.
3. Peptone	25.0 g.
4. NaCl	10.0 g.

Preparation:

(1) Boil 2.5 liters of water with 1250 kg. finely chopped beef, 25.0 g. peptone and 10.0 g. NaCl in streaming steam.
(2) Make slightly alkaline by the addition of soda.

Sterilization: Digest in the steamer until the meat extract is germ free.

Use: Tetanin production. When the medium has cooled following digestion, inoculate and replace the air in the container by hydrogen.

Reference: Kitasato and Weyl (1890 p. 405).

718. Jablon and Pease's Liver Peptone Medium

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.
4. Liver (rabbit, beef or human)	

Preparation:

(1) Dissolve 2 and 3 in 1 by boiling 30 minutes.
(2) Neutralize to phenolphthalein.
(3) Add 20.0 cc. of a normal NaOH solution. The reaction must be a minus 2.
(4) Autoclave for 15 minutes at 115°.
(5) Filter.
(6) Tube in 10.0 cc. lots.
(7) Add approximately 1.0 g. of human, beef or rabbit liver to each tube.
(8) Incubate 3 days to test sterility.
(9) The final reaction should be neutral or slightly alkaline.

Sterilization: Autoclave for 15 minutes at 115°C.

Use: Enrichment medium for streptococci and anaerobes, from war wounds, *Bacillus Welchii*, *Bacillus oedematiens*, *B. sporogenes*, *B. putrificus* and *B. tetani*.

References: Jablons and Pease (1918 p. 1073), Harvey (1921–22 p. 97).

719. Deycke's Albumin Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Alkaline albumin	25.0 g.
3. NaCl	10.0 g.
4. Peptone	10.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Neutralize by adding HCl drop by drop, using litmus paper as an indicator.
(3) Add 2.0% of a soda solution containing 2 parts water to one part soda.
(4) Filter.

Sterilization: Method not given.

Use: Enrichment medium for cholera vibrio.

Reference: Deycke (1893 p. 245).

720. Harvey's Egg Peptone Solution

Constituents:

1. Distilled water	1100.0 cc.
2. NaCl	5.0 g.
3. Peptone	10.0 to 20.0 g.
4. Egg	100.0 cc.
5. Na_2CO_3 (anhydrous 6.5% soln.)	200.0 cc.

Preparation:

(1) Mix the contents of whole eggs with an equal volume of water. (100.0 cc. egg, 100.0 cc. distilled water.)
(2) Add an equal volume of 6.5% anhydrous Na_2CO_3 solution to (1).
(3) Store sterile (2) as a stock solution.
(4) Dissolve 2 and 3 in 1 liter of distilled water.
(5) Mix 1 volume of (2) with 9 volumes of (4).

Sterilization: Sterilize (2) and (5) by heating for one hour at 100°C.

Use: Enrichment of *V. cholera.*

Reference: Harvey (1921–22 p. 86).

721. Weissenbach's Albumin Peptone Solution

Constituents:

1. Distilled water	
2. Peptone (Chapoteaut)	4.0 g.
3. NaCl	0.5 g.
4. Glucose	0.2 g.
5. Egg white	

Preparation:

(1) Dissolve 2 and 3 in 100.0 cc. distilled water.
(2) Make slightly alkaline to litmus.
(3) Autoclave at 120°C. for 15 minutes.
(4) Filter and add 0.2 g. glucose to the filtrate.
(5) Add slowly one volume of egg white to three volumes distilled water. Shake but do not cause the mixture to foam.
(6) Add 0.5 cc. of a 10.0% soda solution to each 100.0 cc. of (5).
(7) Autoclave (6) at 115° for 15 minutes.
(8) Filter while hot.
(9) Mix equal volumes of (4) and (8).
(10) Tube in 5.0 cc. lots.

Sterilization: Autoclave the tubes at 110°C. for 15 minutes.

Use: Cultivation of streptococci from wounds.

Reference: Weissenbach (1918 p. 491).

722. Sherman and Albus' Basal Yeast Fat Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone (1.0%)	10.0 g.
3. Yeast (dry) (1.0%)	10.0 g.
4. Butterfat (1.0%)	10.0 g.
5. Agar (0.1%)	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 1.0% of one of the added nutrients in 1.
(2) Adjust to pH from 6.5 to 7.0.

Sterilization: Not specified.

Use: Cultivation of lactobacilli. The authors report that the 0.1% agar is not essential but seems to favor the growth of some organisms.

Added nutrients: The authors added 1.0% of any desired fermentable carbohydrate.

Variants: Lard and mineral oil were substituted for butterfat.

Reference: Sherman and Albus (1922 p. 17).

723. Weiss and Wilkes-Weiss' Egg Solution

Constituents:

1. Water	200.0 cc.
2. Peptone (Difco) (1.0%)	2.0 g.
3. Sodium phosphate (0.2%)	0.4 g.
4. Agar (0.2%)	0.4 g.
5. Eggs	2

Preparation:

(1) Remove the contents of two fresh eggs with sterile precautions.
(2) Dissolve 2, 3 and 4 in 1.
(3) Emulsify (1) in (2).
(4) Adjust to pH = 7.8 and bring slowly to a boil, stirring frequently.
(5) Tube in spirochete tubes.

Sterilization: Sterilize in the Arnold for 30 minutes and seal with 1 cm. of sterile yellow petrolatum while the tubes are still hot.

Use: Cultivation of *Spirochaeta pallida*.
Reference: Weiss and Wilkes-Weiss (1924 p. 222).

724. Kelser's Blood Infusion Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Beef blood	500.0 g.
3. Peptone	15.0 g.
4. NaCl	7.5 g.

Preparation:
(1) Allow beef blood to clot in covered vessel. Let stand in refrigerator to allow serum to separate from clot.
(2) Remove clot and chop finely in chopping machine.
(3) Mix serum and ground clot. Weigh.
(4) Add two volumes of distilled water.
(5) Boil gently for 5 minutes.
(6) Filter thru cheese cloth. Take residue and put thru fruit press to extract as much fluid as possible, lining press with towel or heavy cloth so that pulp does not pass thru.
(7) Discard the residue.
(8) Boil fluid, skimming off coagulated proteins.
(9) Add concentrated CH_3COOH (0.5 cc. per L) to cause flocculation and continue to boil 5 minutes.
(10) Filter—first thru absorbent cotton and two filter papers.
(11) Ascertain volume and add 0.5% NaCl, and 1.0% peptone. Heat to get into solution.
(12) Neutralize with NaOH.

Sterilization: Sterilize in autoclave ½ hour under 12 pounds pressure.

Use: Nutrient medium for pathogenic forms. The author reported that glucose aided the growth of some organisms.

Variants:
(a) Kelser added 0.25% glucose.
(b) Harvey prepared a medium, boiling the water, clot and serum mixture (see (5) above) for 10 minutes instead of 5, specified glacial acetic acid, steamed the peptone NaCl mixture (see (11) above) for 45 minutes, and adjusted the reaction to a definite pH value or faintly alkaline to litmus, or 1.0% acid to phenolphthalein.
(c) Harvey prepared a medium by heating the water, clot serum mixture (see (4) above) at 50°C. for 20 minutes and then boiling 10 minutes instead of 5, boiled 5 minutes after the addition of the glacial acetic acid (see (9) above), steamed the peptone NaCl mixture (see (11) above) for 45 minutes instead of heating only until solution was complete and adjusted the reaction to a definite pH value, faintly alkaline to litmus or 1.0% acid to phenolphthalein. After adjusting the reaction the medium was steamed for 30 minutes, filtered while hot thru wet thick filter paper and tubed.

References: Kelser (1916 pp. 615, 616), Harvey (1921–22 pp. 69, 76).

725. Wiens' Blood Peptone Solution (Klimmer)

Constituents:

1. Water	1000.0 cc.
2. Peptone (10.0%)	100.0 g.
3. Glucose (1.0%)	10.0 g.
4. Blood	100.0 cc.

Preparation:
(1) Prepare a 10.0% peptone solution in water.
(2) Make slightly alkaline.
(3) Dissolve 1.0% glucose in (2).
(4) Tube in 10.0 cc. quantities.
(5) Add 1.0 cc. of blood to each tube.

Sterilization: Not specified.
Use: Cultivation of pneumococci.
Reference: Klimmer (1923 p. 221).

726. Kligler's Peptone Serum Blood Medium

Constituents:

1. Saline	200.0 cc.
2. Serum (horse or rabbit)	100.0 cc.
3. Blood	
4. Peptone (1.0%)	3.0 g.

Preparation:
(1) Dilute horse or rabbit serum 1:2 with saline.
(2) Adjust to pH 7.0.
(3) Add 1.0% peptone (1.0 cc. of a 10.0% solution per 10.0 cc. medium).
(4) Distribute in 3 to 4.0 cc. lots in Wassermann tubes.
(5) Add 0.1 cc. of blood. (For initial cultures, add infected blood. For

subcultures add fresh normal rabbit blood.)

(6) Cover with a layer of paraffin 1.5 cm. high.

Sterilization: Not specified.

Use: Cultivation of *Spironema duttoni*. Incubate for 24 hours at 37°C., and then at room temperature.

Reference: Kligler (1922 p. 215).

727. Hiss' Basal Serum Peptone Solution (Buerger et al.)

Constituents:

1. Distilled water	1000.0 cc.
2. Serum (Beef)	500.0 cc.
3. Peptone	30.0 g.
4. Litmus (Kahlbaum's)	

Preparation:

(1) Mix a liter of distilled water and 500.0 cc. of beef serum.

(2) Flask and steam for 10 minutes.

(3) Dissolve 30.0 g. of peptone in a little water (100 to 150.0 cc.) by heating over a small flame.

(4) Filter (3) and cool.

(5) Cool (2) and add to (3).

(6) Add one of the added nutrients and sufficient Kahlbaum's litmus to give the desired color.

Sterilization: Sterilize for 15 minutes on each of 3 or 4 successive days in streaming steam.

Use: To study fermentation ability of pneumococci, gonococci, streptococci, etc.

Added nutrients and variants:

(a) Buerger added 1.0% inulin.

(b) Ruediger added 5.0 g. NaCl, used 20.0 g. Witte's peptone and prepared the medium as follows:

(1) Dissolve 5.0 g. NaCl, 20.0 g. Witte's peptone and 2.0% inulin or other carbohydrate in a liter of water.

(2) Add 20.0 cc. litmus.

(3) Tube in 2.0 cc. lots.

(4) Sterilize in the autoclave if using inulin; using other carbohydrates sterilize intermittently.

(5) Collect beef serum without special precautions and dilute with an equal volume of water.

(6) Pass (5) thru a Berkefeld filter, flask in 50.0 to 100.0 cc. quantities and heat at 65°C. for 30 minutes on each of 2 successive days.

(7) Add 2.0 cc. of (6) to each tube of (4) under aseptic conditions.

(c) Watabiki added 5.0 g. NaCl, used 10.0 g. Witte's peptone and prepared the medium as follows:

(1) Dissolve 10.0 g. Witte's peptone, 5.0 g. NaCl in a liter of distilled water.

(2) Boil for 30 minutes and filter.

(3) Add 15.0 g. of one of the following materials dissolved in a little water and 20.0 cc. of a 5.0% litmus solution to (2).

mannitol
maltose
glucose
dextrin
sucrose
dulcitol
inulin
lactose
levulose
galactose

(4) Distribute in 5.0 cc. quantities in test tubes.

(5) Sterilize for 30 minutes on each of 3 successive days.

(6) Add 5.0 cc. of fresh horse serum heated to 55 to 60° for 40 minutes to each tube.

(7) Incubate for 24 hours to test sterility.

(d) Park, Williams and Krumwiede prepared the medium as follows:

(1) Dilute serum with 2 or 3 times its volume of distilled water.

(2) Sterilize (1) in the Arnold.

(3) Prepare a 10.0% solution of peptone.

(4) Sterilize (3), method not given.

(5) Prepare a 10.0 or 20.0% solution of any desired carbon source or fermentable material.

(6) Heat (5) in small containers in the Arnold sterilizer for 30 minutes on each of 3 successive days. When using inulin, sterilize in the autoclave.

(7) Add, under aseptic conditions, sufficient (4) to (2) to give a 1.0% peptone concentration.

(8) Add, under aseptic conditions, sufficient (6) to (7) to give a 1.0% concentration of carbon source. (Generally 5.0% glycerol is employed instead of 1.0%.) In routine work with glucose, lactose, sucrose, mannitol and dulcitol it is generally sufficient to add the sugar to the

medium and sterilize in the Arnold for 30 minutes on each of 3 successive days.

(e) Park, Williams and Krumwiede also suggested the following method of preparation:
  (1) Dissolve 20.0 g. of peptone and 20.0 g. of inulin or other carbohydrate in a liter of water.
  (2) Add sufficient litmus to give the desired color.
  (3) Tube.
  (4) Sterilize in the autoclave when using inulin, and on each of 3 successive days when using any other source of carbon.
  (5) When cool add an equal volume of sterile serum to each tube.

References: Buerger (1905 p. 524), Ruediger (1906 p. 756), Watabiki (1909 p. 366), Park, Williams and Krumwiede (1924 p. 124).

728. Emile-Weil's Pleuritic Serum Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl	8.0 g.
3. Peptone	20.0 g.
4. Glucose	6.0 g.
5. Pleuritic serum (human)	500.0 cc.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Mix one part of human pleuritic serum to 2 parts (1).

Sterilization: Not specified.

Use: Cultivation of *Bacillus leprae.*

Reference: Emile-Weil (1905 p. 798).

729. Hiss' Basal Ascitic Fluid Peptone Solution (Park, Williams and Krumwiede)

Same as medium 727, Variant (e) except ascitic fluid is substituted for serum.

730. Abbott's Bile Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Bile (ox, dried)	100.0 g.
3. Peptone	10.0 g.

Preparation:
(1) Dissolve 2 and 3 in 1.
(2) Tube.

Sterilization: Not specified.

Use: Enrichment of typhoid bacilli.

Reference: Abbott (1921 p. 521).

731. Stitt's Bile Peptone Solution

Constituents:

1. Bile	1000.0 cc.
2. Peptone (1.0%)	10.0 g.

Preparation:
(1) Secure ox bile from the abattoir or human bile from patients undergoing bladder drainage in hospitals.
(2) Dissolve 1.0% peptone in (1).
(3) Tube in 10.0 cc. quantities.

Sterilization: Method not given.

Use: Enrichment of typhoid bacilli.

Reference: Stitt (1923 p. 47).

732. Boudeille's Bile Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	60.0 g.
3. Glucose	
4. Bile	

Preparation:
(1) Dissolve 2 in 1.
(2) Adjustment of reaction not given.
(3) Distribute in 5.0 cc. lots in tubes.
(4) Prepare a 5.0% glucose solution.
(5) Add 2.0 cc. of (4) to each tube of (3).
(6) Add various amounts of bile to each tube. (0.0 to 4.0 cc. Add water to other tubes to have an equal volume in each tube.)

Sterilization: Sterilize in the autoclave.

Use: To study effect of bile on colon bacilli. The presence of bile inhibited the fermentation of glucose by the colon bacilli. Enrichment medium for typhoid bacilli, differentiation between streptococci, enterococcus and diplococcus.

Variants:

(a) Tribondeau and Dubreuil and Harvey autoclaved a mixture of 1.0% glucose and 1.0% peptone in beef bile at 120° for 20 minutes. The solution was filtered thru wet filter paper while hot, tubed with fermentation tubes and sterilized at 115° for 20 minutes. This medium was used as an enrichment medium for the typhoid bacilli. Paratyphoid bacilli produced gas while typhoid bacilli did not.

(b) Weissenbach used the following solution to differentiate between streptococci, enterococci and diplococci. Enterococci and diplococci clouded the medium while streptococci showed no growth:

1. Water	1000.0 cc.
2. Peptone	40.0 g.
3. NaCl	5.0 g.
4. Glucose	2.0 g.
5. Bile (beef)	250.0 cc.

References: Boudeille (1912 p. 783), Tribondeau and Dubreuil (1918 p. 131), Weissenbach (1918 p. 560), Harvey (1921–22 p. 89).

733. Jackson and Melia's Bile Peptone Solution

Constituents:

1. Bile (sterile fresh ox)	1000.0 cc.
2. Peptone	10.0 g.
3. Lactose	10.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Tube in fermentation tubes.

Sterilization: Method of sterilization of ox bile or final medium not given.

Use: Enrichment medium in water analysis. Intestinal forms produced gas.

Variants:

(a) Obst specified that the bile should never be used after one week following collection.

(b) Bunker used 20.0 to 50.0 g. of dried bile in a liter of water instead of 1000.0 cc. of fresh bile.

(c) Ball specified the use of a 1.0% bile solution instead of fresh bile.

(d) Ritter used a mixture of 500.0 cc. of water and 500.0 cc. ox bile instead of fresh ox bile.

(e) Tanner and Heinemann specified that the final medium be sterilized in the Arnold sterilizer on each of 3 successive days.

(f) Bacto Lactose Peptone Bile, dehydrated, is a preparation of 50 parts Bacto ox Bile, 10 parts Bacto peptone and 10 parts Bacto lactose. Dissolve 70.0 g. of this preparation in a liter of distilled water. Sterilize in the usual manner.

References: Jackson and Melia (1909 p. 194), Obst (1916 p. 76), Bunker (1916 pp. 85–86), Ball (1919 p. 81), Ritter (1919 p. 609), Tanner (1919 p. 59), Heinemann (1922 p. 35), Park, Williams and Krumwiede (1924 p. 132), Digestive Ferments Co. (1925 p. 13).

734. Olszewski and Köhler's Bile Peptone Solution

Constituents:

1. Water	12,000.0 cc.
2. Peptone	75.0 g.
3. NaCl	50.0 g.
4. Bile	500.0 cc.
5. Litmus solution	600.0 cc.
6. Lactose	75.0 g.

Preparation:

(1) Dissolve 75.0 g. peptone and 50.0 g. NaCl in 500.0 cc. of bile by boiling.

(2) Filter.

(3) Dissolve 75.0 g. lactose in 600.0 cc. litmus solution prepared according to Kubel and Tiemann with Kahlbaum's litmus (method or reference not given).

(4) Boil (3) for 15 minutes.

(5) Filter and add NaOH or soda solution to neutralize if necessary.

(6) Mix sterile (2) and sterile (5) before use.

(7) Tube.

Sterilization: Sterilize (2) and (5) separately and mix. Sterilize the mixture. Method of sterilization not given.

Use: Water analysis. Presumptive test for *B. coli*. Add 10 parts of the water under investigation to 1 part of the medium. *B. coli* turned the medium red, other forms being inhibited.

Reference: Olszewski and Köhler (1922 p. 307).

735. Muer and Harris' Brilliant Green Lactose Peptone Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Ox gall (dried)	50.0 g.
3. Peptone	10.0 g.
4. Lactose	10.0 g.
5. Brilliant green	0.1 g.

Preparation:

(1) Heat 1 liter of distilled water in a double boiler until the water in the outer vessel boils.

(2) Add 50.0 g. of dried ox gall and 10.0 g. peptone to (1), stirring until solution is complete.

(3) Boil for one hour.
(4) Remove from the flame and add 10.0 g. of powdered lactose.
(5) Filter thru cotton flannel until clear.
(6) To each liter of the filtrate add 10.0 cc. of a 1.0% solution of brilliant green.
(7) Tube.

Sterilization: Sterilize in the autoclave for 15 minutes at 15 pounds pressure.

Use: Presumptive test for *B. coli* in water analysis. Author reported that *B. coli* produced gas in this medium. Other forms were inhibited, especially *B. welchii* and other anaerobes.

References: Muer and Harris (1920 p. 875), Levine (1921 p. 112), Park, Williams and Krumwiede (1924 p. 132).

736. Conradi's Bile Peptone Solution

Constituents:

1. Bile (fresh beef)	900.0 cc.
2. Peptone (Witte)	100.0 g.
3. Glycerol	100.0 g.

Preparation:

(1) Mix 1, 2 and 3.
(2) Place the unfiltered sterile (1) in 2.0 or 3.0 cc. quantities in sterile glass tubes 9.0 cm. high and 18 mm. in diameter with rubber stoppers.

Sterilization: Sterilize (1) in streaming steam for 2 hours. Heat (2) at 100°C. for 30 minutes.

Use: Enrichment and isolation of typhoid bacilli from patient's blood. About 0.5 to 2.0 cc. of the patient's blood is added to each tube of medium.

Variants: Roddy and Stitt used 2.0% peptone.

References: Conradi (1906 p. 58), (1906 p. 1655), Roddy (1917 p. 42), Harvey (1921–22 p. 89), Stitt (1923 p. 47), Klimmer (1923 p. 214).

737. Vierling's Whey Peptone Solution

Constituents:

1. Distilled water	350.0 cc.
2. Skim milk powder	30.0 g.
3. Physiological salt solution	150.0 cc.
4. Peptone	0.02 to 0.03 g.
5. Kahlbaum's litmus solution	20.0 to 30.0 cc.

Preparation:

(1) Dissolve 30.0 g. of skim milk powder in 200.0 cc. distilled water by heating on a water bath. (Milk powder is prepared by evaporating skim milk to $\frac{1}{4}$ its volume in a vacuum, powdered, and pass the powder thru a hot stream of air to dry.)
(2) Add 4.0 cc. of an 18.0% $CaCl_2$ solution to (1) and heat in the steamer for 40 minutes to precipitate the casein.
(3) Add 2.0 cc. N/1 soda solution and heat for 25 more minutes.
(4) Filter thru a folded filter, pressing out the whey by means of cloth.
(5) If not clear filter thru moistened filter paper shreds using suction. The whey will not become bright and clear unless it is allowed to stand in the ice box for 3 to 5 days.
(6) Add 150.0 g. distilled water and 150.0 cc. physiological salt solution making the volume to 500.0 cc.
(7) Dissolve 0.02 or 0.03 g. Witte peptone in (6).
(8) Sterilize for 30 minutes. A turbudity is formed which settles out by standing in the ice box over night. Filtering is unsatisfactory.
(9) Add 20.0 to 30.0 cc. sterile Kahlbaum's litmus tincture to (8) by means of a sterile pipette.
(10) Standardize the tint by the addition of N/10 soda solution or lactic acid. The medium will become somewhat bluer by sterilization so a rather red tinge is to be desired.
(11) Prepare the tubes in which the whey is to be distributed by adding about 10 mg. $CaCO_3$ to each tube and heating for 1 hour, dry heat at 120 to 140°C. The tubes should be of Jena glass or tubes that have been used a great deal. The $CaCO_3$ tends to neutralize the acids formed from galactose and glucose which have come from hydrolysed lactose. It does not injure the reddening or the whey. The tubes may be used without the $CaCO_3$.
(12) Distribute into 5.0 cc. lots into sterile test tubes.

Sterilization: Sterilize for 5 minutes (method not given).

Use: General culture medium.

Reference: Vierling (1922 p. 94), Klimmer (1923 p. 209).

738. Park, Williams and Krumwiede's Whey Peptone Solution

Constituents:

1. Whey	1000.0 cc.
2. Peptone (2.0%)	20.0 g.
3. NaCl (0.5%)	5.0 g.

Preparation:

(1) Allow 3.0 quarts of unpasterized milk to stand over night.
(2) Remove the cream the next morning.
(3) Heat the milk over a flame and stir until ready to boil.
(4) Add 6.0 cc. of glacial acetic acid (0.2%) to coagulate the casein.
(5) Strain thru cheese cloth.
(6) Add 2.0% peptone and 0.5% NaCl.
(7) Boil, stirring to dissolve the peptone and salt.
(8) Adjust the reaction to 0.5% acid to phenolphthalein.
(9) Cool to 45°C.
(10) Add the whites of 3 eggs to clear.
(11) Filter thru cotton and paper.

Sterilization: Sterilize in the autoclave at 15 pounds for 30 minutes.

Use: General culture medium.

Reference: Park, Williams and Krumwiede (1924 p. 134).

739. Cunningham's Peptone Whey Solution

Constituents:

1. Whey	1000.0 cc.
2. Peptone (1.0%)	10.0 g.
3. NaCl (0.5%)	5.0 g.
4. Andrades indicator (1.0%)	10.0 cc.

Preparation:

(1) Warm 2 liters of clean, fresh skim milk in a large pot at 37°C.
(2) Add sufficient rennet to curdle.
(3) Allow to settle for 10 minutes.
(4) Break the curd into large pieces by means of a stirring rod.
(5) Heat to 80°C. to contract the clot and to express the whey.
(6) Strain thru a cheese cloth.
(7) Add 1.0% peptone and 0.5% NaCl.
(8) Steam for 30 minutes.
(9) Neutralize to turmeric paper.
(10) Steam for one hour.
(11) Filter thru paper until clear.
(12) Add 1.0% Andrades indicator.

Sterilization: Sterilize intermittently in steam.

Use: Bacteriologic examination of milk and dairy products.

Reference: Cunningham (1924 p. 101).

740. Kan-Ichiro Morishima's Nutrose Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Nutrose	2.5 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.

Preparation:

(1) Dissolve 2 in 1.
(2) Inoculate with a culture of *B. coli*.
(3) Incubate for 24 hours at 37°C.
(4) Autoclave.
(5) Filter.
(6) Dissolve 3 and 4 in the filtrate.
(7) Adjust to pH = 7.0 to 7.1.

Sterilization: Autoclave at 15 pounds for 15 minutes.

Use: Cultivation of typhoid bacilli.

Reference: Kan-Ichiro Morishima (1921 p. 277).

741. Elser and Huntoon's Basal Nutrose Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Nutrose	10.0 g.
3. Peptone (Witte)	10.0 g.
4. NaCl	5.0 g.
5. Litmus solution (Merck's)	5.0 to 7.5 cc.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Add 5.0 to 7.5 cc. of a watery solution of Merck's highly sensitized litmus.
(3) Prepare 100.0 cc. of a 10.0% solution of one of the added nutrients in distilled water.
(4) Mix sterile (2) with sterile (3).
(5) Tube in sterile tubes.
(6) Incubate for 3 days to detect accidental contamination.

Sterilization: Sterilize (2) in the usual manner (method not given). Sterilize (3) at 100°C. for 10 minutes.

Use: To study fermentation by meningococci. Other investigators used similar media for the cultivation of diphtheria bacilli.

Added nutrients:

(a) The author added 1.0% of one of the following:

glucose
galactose
levulose
lactose
maltose
sucrose
mannitol
dulcitol
inulin
dextrin

The following solutions have been described as Thiel's Nutrose solutions and were used to study fermentation ability of diphtheria and pseudo diphtheria bacilli.

(b) Roux and Rochaix used the basic solution with 50.0 cc. of Kahlbaum's litmus solution instead of 5.0 to 7.5 cc. Merck's without any additions.

(c) v. Przewaski used the same basic solution with 5.0 cc. of Kahlbaum's litmus solution and added 20.0 cc. of a 1.0% crystalline soda solution. To this basic solution he added 1.0% of one of the following:

glucose
lactose
levulose
mannose
inulin
mannitol
dulcitol

References: Elser and Huntoon (1909 p. 404), Roux and Rochaix (1911 p. 247), v. Przewaski (1912 p. 13), Klimmer (1923 p. 222).

742. Loeffler's Malachite Green Nutrose Solution

Constituents:

1. Distilled water....	1000.0 cc.
2. Peptone (2.0%).....	20.0 g.
3. Nutrose (1.0%)....	10.0 g.
4. Lactose (5.0%)....	50.0 g.
5. Malachite green (2.0% solution)....	30.0 to 100.0 cc.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Add 10.6 cc. of normal NaOH and cool to about 50°C.
(3) Add from 30.0 to 100.0 cc. of a 2.0% malachite green solution.

Sterilization: Not given.

Use: Enrichment of colon typhoid group.

Variants:

(a) The author added 1.0% glucose.

(b) Klimmer prepared the medium as follows:

(1) Dissolve the peptone and glucose (0.0 or 1.0%) in 800.0 cc. of distilled water.
(2) Add 10.6 cc. of normal KOH.
(3) Dissolve 10.0 g. nutrose in 200.0 cc. of hot distilled water and add the lactose.
(4) Add (3) to (2).
(5) Flask in 100.0 cc. quantities.
(6) Sterilize on each of 3 successive days for 10 minutes in streaming steam.
(7) Add 3.0 cc. of a 2.0% solution of 120 Höchst malachite green to each 100.0 cc. lot.
(8) Tube in 3.0 or 4.0 cc. quantities.

(c) Klimmer prepared the medium as in variant (b) above, but omitted the lactose (using 1.0% glucose) and only added 1.0 cc. of the 2.0% malachite green per 100.0 cc. of medium instead of 3.0 cc.

References: Loeffler (1906 pp. 289–295), Klimmer (1923 p. 213).

743. Seliber's Basal Casein Peptone Solution

Constituents:

1. Distilled water............	1000.0 cc.
2. K_2HPO_4..................	1.0 g.
3. $MgSO_4$....................	0.3 g.
4. NaCl......................	0.1 g.
5. $CaCl_2$....................	0.1 g.
6. Peptone...................	10.0 g.
7. Casein....................	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Neutralize (indicator not specified) and add a slight excess of NaOH.
(3) Add 5.0 g. casein to (2).
(4) Heat on a salt water bath, shaking thoroughly to obtain a homogenous mixture of casein.
(5) Filter or decant sterile (4).
(6) Dissolve 1.0% of any desired carbohydrate, alcohol, etc. in (5).
(7) Tube.

Sterilization: Sterilize (4) at 115°C. for 15 minutes. Sterilize the media after being tubed in the steamer.

Use: Detection of fermentation by coagulation of casein.

Variants: The author added a trace of $FeCl_3$.

Added nutrients: The author added 1.0% of any carbohydrate, alcohol, etc.

References: Seliber (1914 p. 640), Harvey (1921–22 p. 96).

744. Albus and Holm's Milk Yeast Medium (Medium Y)

Constituents:

1. Water	1000.0 cc.
2. Yeast (1.0%)	10.0 g.
3. Peptone (1.0%)	10.0 g.
4. Lactose (1.0%)	10.0 g.
5. Milk (Skim)	10.0 cc.
6. Brom cresol purple (5.0% alcoholic solution)	0.5 cc.
7. Sodium recinolate	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Divide into four lots and to three add various quantities of sodium recinolate, keeping one lot as a control. The surface tension was determined by the weight drop method following sterilization.

Sterilization: Method not given.

Use: To determine the effect of surface tension on growth of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus.* The authors reported that *L. bulgaricus* was inhibited at a surface tension lower than 40 dynes, while *L. acidophilus* exhibited good growth in the same medium, depressed to 36 dynes.

Variants: The authors used sodium taurocholate and sodium glycocholate instead of sodium recinolate to lower the surface tension.

Reference: Albus and Holm (1926 p. 14).

745. Heller's Urine Peptone Solution

Constituents:

1. Urine	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.

Preparation:

(1) Secure human urine as near average specific gravity as possible.

(2) Make weakly alkaline with soda. End point indicated by precipitation of salts.

(3) Filter.

(4) Add peptone and NaCl.

(5) Boil.

(6) Filter.

(7) Tube.

Sterilization: Sterilize in streaming steam.

Use: General culture medium. The author reported growth of a number of molds, saprophytic and parasitic bacteria. Suggested as a substitute for beef infusion.

Variants:

(a) The color and some inhibitory substances may be removed by animal charcoal.

(b) Various carbon sources may be added if desired.

Reference: Heller (1890 p. 893).

746. Piorkowski's Urine Peptone Solution

Constituents:

1. Urine	1000.0 cc.
2. Peptone	5.0 g.

Preparation:

(1) Dissolve 5.0 g. of peptone in 100.0 cc. urine and distribute in 100.0 cc. lots.

(2) Steam for 15 minutes in a steamer.

(3) Filter.

(4) Distribute into 10.0 cc. lots.

Sterilization: Sterilize for 10 to 15 minutes on each of 2 days using the fractional method.

Use: Differentiation of *Bact. coli* and *Bacillus typhi abdomin.*

Reference: Piorkowski (1896 p. 687).

747. Khouvine-Delaunay's Cellulose Fecal Infusion Solution

Constituents:

1. Water	850.0 cc.
2. Fecal infusion	250.0 cc.
3. Cellulose (Berzelius paper)	10.0 g.
4. Peptone (pancreatic)	1.0 g.
5. NaCl	1.0 g.
6. K_2HPO_4	1.0 g.
7. $CaCO_3$	2.0 g.

Preparation:

(1) Thoroughly mix 2 parts fecal material with 8 to 10 parts distilled water.

(2) Filter thru a Laurent filter.

(3) Sterilize (2) for 15 minutes at 110°C.

(4) Allow the precipitate from (3) to settle and use the clear supernatant fluid.

(5) Dissolve 4, 5, 6 and 7 in 850.0 cc. of water and 250.0 cc. of (4). Add 10.0 g. of cellulose (Berzelius paper).

Sterilization: Final sterilization not given.

Use: Cultivation of an anaerobic cellulose digester from the human intestine.

Reference: Khouvine and Delaunay (1922 p. 922).

SUBGROUP I-C. SECTION 13

Liquid media or basal solutions containing peptone (or other commercial digest), and an extract or infusion of animal origin. (Plant derivatives may also be present.)

A_1.* Employed as basal solutions with the addition of other materials.
B_1. Not containing additional organic materials.
C_1. Infusions specified.
  Committee A. P. H. A. (1899) Basal Infusion Broth 748
  Heinemann's Basal Sugar Free Infusion Broth 749
  Hopkins and Lang's Basal Veal Infusion Broth 750
  Harvey's Basal Carbonate Infusion Broth 751
  Davis and Ferry's Basal Infusion Broth 752
C_2. Extracts specified.
  Grimberg's Basal Carbonate Extract Broth 753
  Committee A. P. H. A. (1917) Basal Extract Broth 754
  Percival's Basal Extract Broth 755
  Rogers, Clark and Evans' Basal Extract Broth 756
  Stern's Basal Fuchsin Sulphite Broth 757
  Winslow, Rothberg and Parsons' Basal Extract Broth 758
  Elser and Huntoon's Basal Nährstoff Heyden Broth 759
  Stutzer's Basal Nitrate Extract Broth 760
  Bacto (Dehydrated) Basal Extract Broth 761
C_3. Whether infusion or extract employed not specified.
  Thoinot and Masselin's Basal Bouillon 762
  Klecki's Basal Bouillon 763
  Tanner's Basal Carbonate Bouillon 764
B_2. Containing additional organic materials.
C_1. Infusions specified.
  Wolf's Basal Casein Digest Infusion Broth (Kahn) 765
  Torrey and Buckell's Basal Ascitic Fluid Infusion Broth 766
C_2. Extracts specified.
  Symmers and Wilson's Basal Ascitic Fluid Extract Broth 767
  Holman's Basal Serum Extract Broth 768
C_3. Whether infusions or extracts employed, not specified.
D_1. All additional organic material of known chemical composition.
  MacConkey's Basal Bile Salt Iodide Bouillon 769
  Klimenko's Basal Glycerol Bouillon 770
D_2. Additional organic material of unknown chemical composition present.
E_1. Ascitic fluid used.
  Cantani's Basal Ascitic Fluid Bouillon 771
  Buerger's Basal Ascitic Fluid Bouillon 772
  Akatsu's Basal Ascitic Fluid Bouillon 773
E_2. Serum used.
  Hiss' Basal Serum Bouillon 774
  Holman's Basal Serum Bouillon 775
A_2. Employed as complete media.
B_1.* Not containing additional organic materials.
C_1. Prepared dehydrated media.
  Ragit's Dehydrated Broth 776
  Bacto Dehydrated Broth 777
  Harvey and Iyengar's Dehydrated Broth 778
C_2. Media prepared from constituents.
D_1.* Infusions specified.
E_1.† Beef infusions employed.
F_1. Containing peptone.
  Dunham's Infusion Broth 779
  Jensen's Nitrate Infusion Broth 780
  Davis and Ferry's Nitrate Infusion Broth 781
  Loeffler's Infusion Broth (Roux and Rochaix) 782
  Zielleczky's Phenolphthalein Infusion Broth 783
  Muller's Indicator Infusion Broth 784
F_2. Not containing peptone.
  Robinson and Rettger's Infusion Opsine Solution 785

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* See next column for A_2.

* See next page for B_2 and D_2.

† See next page for E_2 to E_5.

E$_2$. Veal infusion employed.
- Warden, Connell and Holly's Veal Infusion Broth 786
- Schoenholz and Meyer's Veal Infusion Broth 787
- Bunker's Veal Infusion Poly Peptide Solution 788

E$_3$. Infusions of animal organs employed.
- Krasnow's et al. Sugar Free Veal Broth 789
- Cesaris-Demel's Liver Infusion Broth. 790
- Owen, Martin & Pitt's Trypsin Beef Tea 791
- Kligler's Heart Infusion Broth 792
- Harvey's Heart Infusion Broth 793
- Park, Williams and Krumwiede's Heart Infusion Broth 794
- Wroblewski's Suprarenal Capsule Infusion Broth 795
- Harvey's Organ Infusion Broth 796
- Kligler's Blood Clot Infusion Broth. 797

E$_4$. Fish Infusion employed.
- Hunter's Fish Infusion Broth 798
- Harvey's Fish Infusion Broth 799

E$_5$. Infusions other than E$_1$ to E$_4$ employed.
- Richardson's Mucosa Infusion Broth. 800
- Boyer's Bone Infusion Broth 801
- Ball's Animal Infusion Broth 802

D$_2$. Extracts specified.

E$_1$. Containing nitrates.
- Stutzer's Nitrate Extract Broth 803
- Davis and Ferry's Nitrate Bouillon Extract Broth 804

E$_2$. Not containing nitrates.

F$_1$. Sodium chloride added.
- Debrand's Extract Broth 805
- Berman and Rettger's Extract Proteose Solution 806
- Guth's Selenium Extract Broth 807
- Berman and Rettger's Salt Extract Broth 808
- Berman and Rettger's Trypsinized Extract Broth 809
- Olszewski and Köhler's Trypsinized Extract Broth 810
- Bacto Cooledge Broth (Dehydrated). 811

F$_2$. Sodium chloride not added.
- Heinemann's Bouillon 812
- Wyant's Bouillon Cube Broth 813

D$_3$. Whether infusions or extracts employed not specified.

E$_1$. Nitrates added.
- Heinemann's Nitrate Broth 814

E$_2$. Nitrates not added.
- Makgill's Indicator Bouillon 815
- Thoinot's Arsenious Acid Bouillon 816
- Omeliansky's Dilute Bouillon 817
- Muller and Malvoz's Iodine Bouillon. 818
- Fremlin's Ammonium Sulphate Bouillon 819
- Stitt's Carbonate Bouillon 820
- Dalimier and Lancereau's Opsine Bouillon 821
- Frieber's Trypsinized Bouillon (Comm. S. A. B.) 822

B$_2$. Containing additional organic material.

C$_1$. Infusions specified.

D$_1$.* Veal Infusions employed.

E$_1$. Additional constituents of known chemical composition.
- Wilcox's Glucose Veal Infusion Broth. 823
- Park, Williams and Krumwiede's Glycerol Veal Infusion Broth 824

E$_2$. Additional constituents of unknown chemical composition present.
- Reeser's Potato Veal Infusion Broth. 825
- Mellon's Serum Veal Infusion Broth. 826
- Albert & Kelman's Blood Veal Infusion Broth 827
- Thjötta & Gunderson's Blood Veal Infusion Solution 828
- Robinson and Meader's Liver Veal Infusion Broth 829
- Starin and Dack's Casein Digest Veal Infusion Broth 830
- Bacto Veal Infusion Medium (Dehydrated) 831
- Bruschettinis' Blood Egg Broth (Klimmer) 832

D$_2$.† Liver infusion employed.
- Jackson and Muer's Glucose Liver Infusion 833
- Haslam's Brain Liver Infusion Broth. 834

D$_3$. Heart infusion employed.
- Mueller's Heart Infusion Aminoid Solution 835
- Mueller's Heart Infusion Peptone Solution 836
- Huntoon's Hormone Heart Infusion Broth 837
- MacNoughton's Blood Infusion Broth. 838

D$_4$. Beef (meat) infusions employed.

E$_1$. All additional constituents of known composition.

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* See next page for C$_2$.

† See D$_3$ to D$_5$.

- F_1. Containing additional organic nitrogen.
  - Ficker and Hoffmann's Caffeine Infusion Broth.... 839
  - Ayers and Rupp's Hippurate Infusion Broth.... 840
  - MacConkey's Bile Salt Infusion Broth (Heinemann).... 841
- F_2. Not containing additional organic nitrogen.
- G_1. Carbohydrates added.
  - Smith's Glucose Infusion Broth....841a
  - Mueller's Meat Infusion Broth.... 842
  - Robinson and Rettger's Glucose Infusion Broth.... 843
  - Bronstein & Grünblatt's Indicator Glucose Infusion Broth.... 844
  - Ayers, Rupp and Johnson's Glucose Infusion Broth.... 845
  - Ayers, Rupp and Mudge's Glucose Infusion Broth.... 846
  - Torrey's Brilliant Green Infusion Broth.... 847
  - Torrey's Acetic Acid Infusion Broth. 848
  - Sherman's Lactose Infusion Broth.. 849
  - Harvey's Starch Infusion Broth.... 850
  - Loeffler's Malachite Green Infusion Broth.... 851
  - Duval and Lewis' Inulin Bouillon.... 852
- G_2. Carbohydrates not added.
  - Bulir's Mannitol Infusion Broth.... 853
  - Harvey's Ferric Tartrate Infusion Broth.... 854
  - Harvey's Lead Acetate Infusion Broth.... 855
  - Omeliansky's Formate Infusion Broth.... 856
  - Jordan's Phenol Infusion Broth.... 857
- E_2. Chemical composition of at least one of the additional constituents not known.
- F_1. Egg used.
  - Harvey's Egg Infusion Broth.... 858
  - Olitsky and Kligler's Egg White Infusion Broth.... 859
  - Weiss & Wilkes-Weiss' Egg "Hormone" Broth.... 860
- F_2. Animal tissue used.
  - Kohman's Brain Infusion Medium.. 861
  - Kreidler's Glucose Brain Broth.... 862
  - Kligler's Heart Infusion Solutions.. 863
  - Havens & Taylor's Kidney & Blood Infusion Broth.... 864
  - Robertson's Cooked Meat Medium (Torrey).... 865
- F_3. Animal fluids used.
  - Wade and Manalang's Blood Infusion Broth.... 866
  - Harvey's Ascitic Fluid Infusion Broth. 867
  - Stryker's Serum Infusion Broth.... 868
  - Foster's Serum Infusion Broth.... 869
  - Beach and Hasting's Serum Infusion and Extract Broth.... 870
  - Harvey's Blood Infusion Broth.... 871
- F_4. Other materials of unknown chemical composition used.
  - Park, Williams and Krumwiede's Potato Infusion Broth.... 872
  - Lubinski's Potato Infusion Broth... 873
  - Jurewitsch's Potato Infusion Broth.. 874
  - Olitsky and Gates' Bacteria Infusion Broth.... 875
  - Ogata's Porphyra Infusion Broth.... 876
  - Park, Williams & Krumwiede's Stomach Digest Infusion Broth... 877
  - Hitchen's Yeast Autolysate Blood Solution.... 878
  - Hitchen's Semisolid Glucose Agar (Mulsow).... 879
  - Menten & Manning's Lactose Bile Infusion Solution.... 880
  - Hitchen's Glucose Agar Infusion Solution (Mulsow).... 881
  - Kreidler's Trypsinized Broth.... 882
- D_5. Infusions other than above employed.
  - Richardson's Carcinoma Infusion Broth.... 883
  - Siebert's Horse Meat Infusion Broth. 884
  - Peklo's Potato Horse Meat Infusion Broth.... 885
  - Hida's Horse Meat Infusion Broth.. 886
  - Khouvine's Fecal Infusion Broth... 887
  - Cutler's Blood Clot Infusion Broth (Stitt).... 888
- C_2.* Extracts specified.
- D_1.* All additional constituents of known chemical composition.
- E_1. Containing additional organic nitrogen.
  - Percival's Urea Extract Broth.... 889
  - Stutzer's Dinitro Benzol Extract Broth.... 890
  - Harrison and Vanderleck's Aesculin Extract Broth.... 891
  - Sawin's Glycocholate Extract Broth. 892
  - Olszewski and Köhler's Trypsinized Bile Salt Extract Broth.... 893
  - Frieber's Tryptophane Extract Broth. 894

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* See next page for C_3 and D_2.

E_2. Not containing additional organic nitrogen.
F_1. Carbohydrates added.
G_1. Containing monosaccharides.
Bachmann's Indicator Extract Broth. 895
Rogers, Clark and Evans' Glucose Extract Broth.................... 896
Waksman and Joffe's Glucose Extract Broth............................ 897
Whipple's Glucose Extract Broth.... 898
Johnson's Glucose Phenol Extract Broth............................ 899
G_2. Containing disaccharides.
Weisser's Sucrose Extract Broth.... 900
Koegel's Lactose Extract Broth..... 901
Gottheil's Sucrose Extract Broth.... 902
G_3. Containing polysaccharides.
Gordon's Starch Extract Broth...... 903
La Garde's Dextrin Peptone Solution.... 904
F_2. Carbohydrates not added; alcohols or acids used.
Banning's Glycerol Extract Broth.. 905
Jacobson's Mannitol Extract Broth. 906
Harrison and Vanderleck's Citrate Aesculin Extract Broth............ 907
D_2. One or more of the additional constituents of unknown chemical composition.
E_1. Unknown added constituents of plant origin present.
De Gaetano's Potato Extract Broth (Kamen)......................... 908
Albus and Holm's Medium X....... 909
E_2. Unknown added constituents of animal origin present.
Flint's Serum Extract Broth........ 910
Grace and Highberger's Ascitic Fluid Extract Broth.................... 911
Olszewski and Köhler's Bile Extract Broth............................ 912
Rosenow's Brain Bacto Broth (Haden)......................... 913
Hoffman and Fischer's Nutrose Extract Broth (Heinemann)......... 914
Keim's Honey Extract Broth........ 915
Ataki & Akimato's Sodium Citrate Blood Extract Solution............ 916
Dimitroff's Egg Sea Water Medium.. 917
C_3. Whether extract or infusion employed not specified.
D_1. All additional constituents of known chemical composition.
E_1. Containing additional organic nitrogen.
Löhnis' Urea Bouillon............... 918
Baruchello's Caffeine Bouillon...... 919
Ficker's Crystal Violet Caffeine Bouillon (Bezançon).............. 920
Müller's Lactose Ferro-cyanide Bouillon.......................... 921
E_2. Not containing additional organic nitrogen.
F_1. Monosaccharides employed.
Frost's Glucose Bouillon............ 922
Riva's Glucose Bouillon............. 923
MacConkey's Iodide Glucose Bouillon............................. 924
Kitasato's Formate Glucose Bouillon (Tanner)......................... 925
Heymann's Acetic Acid Glucose Bouillon (Finkelstein)............ 926
Savage's Neutral Red Glucose Bouillon............................. 927
DeKorti's Oleic Acid Glucose Bouillon............................. 928
Plimmer's Tartaric Acid Infusion Broth............................ 929
F_2. Disaccharides employed.
Gage's Lactose Bouillon............ 930
Hall and Ellefson's Gentian Violet Lactose Broth.................... 931
Jouan's Citrate Lactose Bouillon (Besson)......................... 932
F_3. Polysaccharides employed.
Wollman's Starch Bouillon.......... 933
Hölzel's Glycogen Bouillon......... 934
F_4. Carbohydrates not added. Alcohols, organic acids, etc. employed.
Heinemann's Glycerol Bouillon...... 935
Kendall, Day and Walker's Mannitol Bouillon.......................... 936
Giltner's Adonitol Bouillon......... 937
Vincent's Phenol Bouillon........... 938
D_2. Chemical composition of at least one of the additional constituents not definitely known.
E_1. Unknown constituents of plant origin only.
Thjötta and Avery's Yeast Bouillon.. 939
Thjötta and Avery's Tomato Bouillon............................. 940
Thjötta's Pea or Bean Bouillon..... 941
Thjötta and Avery's Potato Bouillon............................. 942
Thjötta's Bacterial Emulsion Bouillon............................. 943
Hitchens' Basal Sugar-Free Agar Solution.......................... 944

Hegner and Becker's Blood Agar Solution . . . 945

E_2.* Unknown constituents of animal origin only.

F_1. Animal tissue or cells employed.

G_1. Blood cells added.

Thjötta and Avery's Blood Cell Bouillon . . . 946

G_2. Heart, kidney or other tissue added.

Smith, Brown and Walker's Tissue Bouillon . . . 947

Orr's Glucose Heart Bouillon . . . 948

Park, Williams and Krumwiede's Meat Medium . . . 949

Kligler's Tissue Infusion Bouillon . . . 950

G_3. Eggs or derivatives added.

Capaldi's Egg Yolk Bouillon . . . 951

Oberstadt's Egg Albumin Bouillon . . . 952

Lipschütz's Egg Albumin Bouillon . . . 953

Kahn's Casein Digest Egg Albumin Bouillon . . . 954

Besredka and Jupille's Egg Bouillon (Besson) . . . 955

Robertson's Alkaline Egg Bouillon . . . 956

Piorkowski's Alkaline Egg Albumin Bouillon . . . 957

F_2.* Animal fluids employed.

G_1. Blood added.

Hibler's Blood Bouillon . . . 958

Dieudonne's Alkaline Blood Bouillon . 959

Orcutt and Howe's Fat Blood Bouillon . . . 960

Fildes' Pepsinized Blood Bouillon . . . 961

G_2. Serum added.

Dunham's Glucose Serum Bouillon . . . 962

Shmamine's Liver Serum Bouillon . . . 963

Besson's Serum Bouillon . . . 964

G_3. Ascitic fluid added.

Veillon's Ascitic Fluid Bouillon . . . 965

Kahn's Casein Digest Ascitic Fluid Bouillon . . . 966

Lyall's Carbonate Ascitic Fluid Bouillon . . . 967

G_4. Bile or other fluids added.

Roddy's Bile Bouillon . . . 968

F_3. Animal secretions or excretions employed.

Mayer's Mucin Bouillon . . . 969

Schloffer's Urine Bouillon . . . 970

Kligler's Nasal Secretion Bouillon . . . 971

E_3. Containing both plant and animal constituents of unknown chemical composition.

Thjötta and Avery's Yeast Ascitic Fluid Bouillon . . . 972

Thjötta and Avery's Yeast Serum Bouillon . . . 973

Thjötta and Avery's Yeast Blood Bouillon . . . 974

Thjötta and Avery's Yeast Blood Cell Bouillon . . . 975

Thjötta and Avery's Yeast Hemoglobin Bouillon . . . 976

Savini and Savini-Castano's Bacteria Blood Bouillon . . . 977

748. Committee A. P. H. A. (1899) Basal Infusion Broth

Constituents:

1. Distilled water	1000.0 cc.
2. Meat	500.0 g.
3. Peptone (1.0%)	10.0 g.
4. NaCl (0.5%)	5.0 g.

Preparation:

(1) Macerate one part finely chopped lean meat with 2 parts distilled water in the ice box for 18 hours, stirring occasionally.

(2) Strain, cold, thru a fine cloth.

(3) Add 1.0% peptone and 0.5% NaCl to the filtrate. Heat until solution is complete.

(4) Add NaOH until the reaction is slightly alkaline (practically neutral) to phenolphthalein.

(5) Heat on a water bath for 30 minutes and boil for 5 minutes over a free flame.

(6) Filter while hot thru paper or cotton and cloth.

(7) Add N/1 HCl to the filtrate to obtain the desired reaction (+1.5).

(8) Add 1.0% of one of the added nutrients.

Sterilization: Sterilize on each of 3 successive days at 100°C.

Use: Used as a culture medium to determine the fermentation of various sugars, alcohols, etc. by bacteria.

Added nutrients and variants:

(a) The authors added 1.0% of any desired sugar.

(b) Committee A. P. H. A. (1905) prepared the medium as follows:

  (1) Infuse 500.0 g. finely chopped lean meat with 1000.0 cc. water for 24 hours in refrigerator.

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* See E_3 and F_3.

(2) Make up volume.
(3) Strain thru cotton flannel.
(4) Add 10.0 g. Witte's peptone.
(5) Dissolve by heating on water bath.
(6) Heat over steam bath 30 minutes.
(7) Restore volume.
(8) Adjust reaction to +1 with phenolphthalein.
(9) Boil 2 minutes over free flame.
(10) Restore volume.
(11) Filter thru absorbent cotton and cotton flannel until clear.
(12) Add 1.0% of any sugar to (11). In case of adding glucose the muscle sugar must be removed by fermentation with *B. coli*.
(13) Sterilize in streaming steam.

(c) Percival prepared the medium as follows:
(1) Chop one pound of lean fat-free beef with a knife or mincing machine.
(2) Place (1) in a porcelain dish or glass beaker and add 1 liter of water.
(3) Allow to soak over night in a cool place.
(4) Strain thru muslin, and boil for one hour.
(5) Filter thru filter paper into a large flask.
(6) Make the volume up to 1 liter.
(7) Add 10.0 g. Witte's peptone and 5.0 g. NaCl to (6).
(8) Neutralize to phenolphthalein.
(9) Steam for 15 minutes.
(10) Neutralize again and adjust the reaction to +10.
(11) Filter into sterile flasks or test tubes.
(12) Add 2.0% of lactose, 2.0% glucose or 0.3% $NaNO_3$.

(d) Besson prepared the medium as follows:
(1) Remove all fat and tendons from beef and chop into small pieces.
(2) Allow 500.0 g. of (1) to macerate with 1000.0 cc. of cold water for 6 hours, or if one wishes to remove the sugar 12 hours at 37°C.
(3) Place in an enamelled pot and bring slowly to a boil.
(4) Boil for ten minutes.
(5) Throw on a thick cloth and press the meat free from juice.
(6) Filter the juice thru moistened paper.
(7) Add 10.0 g. Chapoteaut or Defresne peptone, 5.0 g. NaCl and about 1.0 g. of sodium phosphate.
(8) Boil stirring constantly until solution is complete.
(9) Neutralize or make slightly alkaline to litmus by the addition of soda solution.
(10) Heat at 115 to 117°C. for 5 minutes.
(11) Filter until clear.
(12) Make up to 1000.0 cc. by the addition of distilled water.
(13) Add 2.0 to 4.0% of one of the following: glucose, raffinose, lactose, galactose, mannitol, dulcitol, maltose, levulose or glycerol.
(14) Distribute as desired.
(15) Sterilize at 110 to 115°C. for 20 minutes.

(e) Harvey prepared the meat infusion peptone solution (see Dunham's Meat Infusion Peptone Solution, variant (bb) 779) and added 1.0% glucose, 1.0% lactose, 2.0% starch, 1.0% mannitol or 5.0% glycerol. Harvey also added 5.0 cc. of a 1.0% neutral red solution to meat infusion peptone solution and added 0.5% of any desired sugar.

(f) Stitt prepared the infusion broth as in variant (hh) 779, or extract broth variant (o) 689, and added 1.0 to 2.0% of any desired carbohydrate, alcohol, etc.

(g) Park, Williams and Krumwiede prepared infusion broth as in variant (11) 779, and proceeded as follows:
(1) Adjust the reaction of meat extract of the infusion to pH = 7.0 (slightly alkaline to litmus).
(2) To each liter add a broth culture of *B. coli* or one of its allies.
(3) Incubate for 48 hours.
(4) Sterilize in the autoclave.
(5) Test again for the production of acid or gas before adding carbohydrates.
(6) Sterilize (5). (Method not given.)
(7) Prepare 10.0 or 20.0% solutions of the carbohydrates, alcohols, etc.

(8) Heat (7) in small containers in the Arnold sterilizer for 30 minutes on 3 successive days. (Sterilize the inulin solution in the autoclave.)

(9) Add sufficient of (8) to sterile (6) to give a 1.0% concentration of sugar (under aseptic conditions). generally 5.0% glycerin is used instead of 1.0%.

(10) In routine work with glucose, lactose, sucrose, mannitol and dulcitol it is generally sufficient to add the sugar to the medium and sterilize in the Arnold for 30 minutes on each of 3 successive days.

They also used an extract broth, see variant (Q) 779.

References: Committee A. P. H. A. (1899 p. 77), (1905 p. 107), Percival (1920 p. 56), Besson (1920 p. 31), Harvey (1921–22 pp. 87, 112), Stitt (1923 p. 34), Park, Williams and Krumwiede (1924 p. 123).

749. Heinemann's Basal Sugar Free Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Chopped beef	1.0 lb.
3. Peptone (Witte)	10.0 g.
4. NaCl	5.0 g.
5. $CaCl_2$	6.0 g.

Preparation:

(1) Chop beef fine and soak with water over night.

(2) Add a culture of *B. coli* and incubate the mixture at 37° for 24 hours.

(3) Then heat to 60°C. for two hours.

(4) Raise to boiling point and boil 30 minutes.

(5) Press out meat, and the remaining meat mixed again with water and pressed a second time.

(6) Mix both fluids and bring to 1000.0 cc. volume.

(7) Dissolve 3 and 4 in boiling (6).

(8) Add 5 and boil until the reaction is about 0.3 acid to phenolphthalein. (Requires from 30 to 60 minutes.)

(9) Filter sterile (8) while boiling hot, under aseptic conditions.

(10) Dissolve one of the added nutrients in (9).

(11) Distribute in small sterile Erlenmeyer flasks or sterile Nessler tubes.

Sterilization: Method of sterilization not given. Sterilize in large Erlenmeyer flasks before filtering.

Use: To study the fermentation of sugars, alcohols, etc.

Added nutrients and variants:

(a) The author added 1.0% of one of the following to the basic solution.

glucose
lactose
sucrose
raffinose
inulin
mannitol
salicin

(b) Besemer did not specify the preparation or composition of meat infusion bouillon, and adjusted the reaction to +0.3 Fuller's scale. He added 1.0% of one of the following:

fructose
glucose
galactose
mannose
sorbose
arabinose
xylose
rhamnose
lactose
sucrose
raffinose
amylose
dextrin
inulin
glycerol
erythritol
adonitol
dulcitol
mannitol
sorbitol
amygdalin
maltose

(c) Kan Ichiro Morishima, prepared media containing indicators as follows:

(1) Prepare meat infusion broth (method not given).

(2) Inoculate with *B. coli*, incubate for 24 hours, autoclave and filter.

(3) Add 1.0% peptone and 0.5% NaCl.

(4) Sterilize at 15 pounds for 15 minutes.

(5) To broth add sterile sugar solution in concentration of 1.0% sugar solution prepared by dissolving sugar in sterile water and heating in autoclave for 10 minutes at 10 pounds.

(6) Add 5.0 cc. sterile litmus or 5.0 cc. of 2.0% phenol red and 1.2 cc. of decolorized 1.0% aqueous solution of china blue per 100.0 cc. of broth.

(7) Adjust to pH = 7.0–7.1 by means of phenol red.

(d) Giltner prepared a neutral red medium as follows:

(1) Soak one pound of lean finely chopped beef in 1000.0 cc. of distilled water over night (24 hours).

(2) Strain out the meat juice and make up to 1000.0 cc. with distilled water.
(3) Add a 24 hour broth culture of *B. coli* to (2).
(4) Incubate at 37°C. for 12 to 16 hours—no longer.
(5) Mix 1.0% (10.0 g.) peptone into a thin paste with a little water and add to (4).
(6) Heat in the autoclave for 20 minutes or in the steamer for one hour.
(7) Neutralize to phenolphthalein.
(8) Boil over a free flame for 3 to 5 minutes.
(9) Add 1.0% glucose (or any other desired sugar) and 10.0 cc. of a 0.5% solution of neutral red and stir until the sugar is dissolved.
(10) Filter until clear.
(11) Distribute into fermentation tubes.
(12) Sterilize in the autoclave or in flowing steam.

(e) Baker prepared a Brom thymol blue medium as follows:
(1) Digest meat and water for two hours. Cook. Filter thru absorbent cotton and sterilize in autoclave at 18 pounds for 20 minutes.
(2) Inoculate cold broth with *Bact. saccharolyte* (Rivas), incubate at 37°C. for 48 hours.
(3) Sterilize in Arnold for 20 minutes.
(4) Add peptone and NaCl.
(5) Adjust to pH = 7.0 with bromthymol blue.
(6) Steam for 20 minutes and readjust reaction.
(7) Filter medium.
(8) Add 12.0 cc. per liter medium of 0.2% alcoholic solution of bromthymol blue. Tube.
(9) Sterilize at 18 pounds for 20 minutes.
(10) Add sterile sugar solution under aseptic conditions.

References: Heinemann (1915 p. 221), Besemer (1917 p. 179), Kan-Ichiro Morishima (1921 p. 277), Baker (1922 pp. 301, 302), Giltner (1921 p. 380).

750. Hopkins and Lang's Basal Veal Infusion Broth

Constituents:

1. Veal Infusion	1000.0 cc.
2. Peptone (Witte)	10.0 g.
3. NaCl	5.0 g.

Preparation:
(1) Preparation of veal infusion not specified.
(2) Dissolve 2 and 3 in (1).
(3) Adjust the reaction to +0.3.
(4) Dissolve 1.0% of one of the added nutrients in (3).

Sterilization: Method not given.

Use: To study fermentation.

Variants: The author used 2.0% Witte's peptone instead of 1.0%.

Added nutrients: The author added 1.0% of one of the following:

glucose
lactose
sucrose
salicin
raffinose
mannitol
inulin
soluble starch
arabinose

Reference: Hopkins and Lang (1914 p. 72).

751. Harvey's Basal Carbonate Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. $CaCO_3$	5.0 to 20.0 g.

Preparation:
(1) See Dunham's Meat Infusion Peptone Solution variant (bb) 750 for preparation of broth from water, peptone and beef.
(2) Dissolve 1.0% of one of the added nutrients in (1).
(3) Add from 5.0 g. to 20.0 g. $CaCO_3$ to (2).

Sterilization: Method not given.

Use: To study fermentation.

Added nutrients: The author added 10%. of any desired carbohydrate, alcohol, etc.

Reference: Harvey (1921–22 p. 107).

752. Davis and Ferry's Basal Infusion Broth

Constituents:

1. Water	500.0 cc.

2.	Peptone	10.0 g.
3.	Beef infusion	500.0 cc.

Preparation:

(1) Composition of beef infusion not given. Author also used a 1.0% solution of Liebig's beef extract instead of infusion.

(2) Prepare 500.0 cc. of a 2.0% peptone solution.

(3) Mix (1) and (2).

(4) Dissolve one of the combinations given under added nutrients in (3).

(5) Steam 15 minutes and check the reaction.

(6) Distribute as desired.

Sterilization: Sterilize at 115° for 20 minutes.

Use: Cultivation of *Bact. diphtheriae* and toxin production.

Added nutrients: The authors added one of the following materials or combinations of materials.

(a)	xanthine	0.05 g.
	hypoxanthine	0.05 g.
(b)	glucose amine hydrochloride	2.0 g.
(c)	sodium asparaginate	1.5 g.
(d)	creatin	0.2 g.
	creatinin	0.15 g.
(e)	cystine	0.5 g.
(f)	glutaminic acid hydrochloride	2.5 g.
(g)	glycocoll	0.75 g.
(h)	histidine dichloride	0.5 g.
(i)	leucine	30.0 g.
(j)	tryptophane	
(k)	tyrosine	

Reference: Davis and Ferry (1919 pp. 235, 236).

753. Grimbert's Basal Carbonate Extract Broth

Constituents:

1.	Water	1000.0 cc.
2.	Peptone	3.0 g.
3.	Meat extract (Liebig's)	2.0 g.
4.	$CaCO_3$	10.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Dissolve 3.0% of one of the added nutrients in (1).

(3) Add the $CaCO_3$ to (2).

Sterilization: Method not given.

Use: To study fermentation. Author used Friedländer's pneumobacillus.

Added nutrients: The author added 3.0% of any desired fermentable sugar.

Reference: Grimbert (1895 p. 840).

754. Committee A. P. H. A. (1917) Basal Extract Broth

Constituents:

1.	Distilled water	1000.0 cc.
2.	Beef extract	3.0 g.
3.	Peptone	5.0 g.

Preparation:

(1) Add 3.0 g. of beef extract and 5.0 g. of peptone to a liter of distilled water.

(2) Heat slowly on a steam bath to at least 65°C.

(3) Neutralize to phenolphthalein.

(4) Cool to 25°C. and filter thru paper until clear.

(5) Add 1.0% of one of the added nutrients.

Sterilization: Sterilize in the autoclave at 15 pounds (120°C.) for 15 minutes after the pressure reaches 15 pounds.

Use: To study fermentation of carbohydrates, alcohols, etc., by bacteria.

Added nutrients and variants:

(a) The committee added 1.0% of any desired carbohydrate.

(b) Mudge specified the use of Liebig's meat extract, and used 1.0% Witte's peptone. He neutralized to phenolphthalein, added 1.0% of any desired carbohydrate, tubed and sterilized for 15, 30, 60 or 120 minutes in the autoclave, or 15 minutes in the Arnold on each of 3 successive days.

(c) Committee S. A. B. used the same constituents as Committee A. P. H. A. They specified that the medium might be clarified by the addition of egg and adjusted to pH = 6.6 to 7.4. One per cent of any desired carbohydrate was added.

(d) Tanner and Ball added 1.0% of any desired carbohydrate, alcohol, etc., to variant (b) 695.

(e) Committee A. P. H. A. (1920) adjusted to a faint pink using phenol red as an indicator or neutralize to phenolphthalein if the reaction does not come between neutral and +1.0. The committee used 0.5% carbohydrate instead of 1.0% as in 1917.

Sterilize at 15 pounds for 15 minutes after the pressure reaches 15 pounds, provided the total time of exposure to heat is not more than 30 minutes. Otherwise sterilize a 10.0% watery solution of any desired carbohydrate at 100°C. for 1.5 hours, and add to sterile nutrient broth in sufficient quantity to give 0.5% carbohydrate. Tube and sterilize this mixture at 100°C. for 30 minutes. It is permissible to add sufficient sterile carbohydrate solution to each tube of sterile neutral broth by means of a sterile pipette. Incubate these tubes for 24 hours to test sterility.

(f) Park, Williams and Krumwiede (1924) added 2.0% litmus solution (reagent of highest purity) or 1.0% Kahlbaum's azolitmin to Committee A. P. H. A. (1920) broth. They adjusted the reaction to neutral to phenolphthalein (pH about 8.0) and sterilized for 15 minutes at 15 pounds pressure after the addition of 0.5% of any desired carbohydrate. The tubes were cooled rapidly.

(g) Committee A. P. H. A. (1925). Same medium as Committee A. P. H. A. (1920) except that the reaction is adjusted from pH 6.2 to pH 7.0.

References: Mudge (1917 p. 406), Committee A. P. H. A. (1917 p. 85), Committee S. A. B. (1918 p. 116), Ball (1919 p. 77), Tanner (1919 p. 44), Committee A. P. H. A. (1920 p. 95), Levine (1921 p. 110), Park, Williams and Krumwiede (1924 p. 131), Committee A. P. H. A. (1925 p. 97).

755. Percival's Basal Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Meat extract (Lemco)	10.0 g.
3. Peptone	10.0 g.
4. Sodium bicarbonate	1.0 g.
5. Litmus (Kubel-Tiemann)	

Preparation:

(1) Dissolve 2, 3, 4 and one of the added nutrients in 1.

(2) Add sufficient boiled Kubel-Tiemann's litmus solution to give a violet tinge.

Sterilization: Method not given.

Use: To study fermentation of carbohydrates, alcohols, etc.

Added nutrients and variants:

(a) The author added 1.0% of one of the following:

lactose
sucrose
glucose
dulcitol
adonitol

(b) Harvey prepared the medium as follows:

(1) Add the whites of 2 eggs to 1000.0 cc. water.

(2) Add to the mixture by degrees to make a suspension: Lemco 3 to 5.0 g., peptone 10.0 g., sodium chloride 5.0 g.

(3) Steam or boil 45 minutes.

(4) Filter while hot thru well-wetted, thick filter paper, or thru 2 layers of absorbent cotton wool.

(5) Bring the volume up to 1000.0 cc. by the addition of water.

(6) Estimate and adjust to a definite pH value or faintly alkaline to litmus or 1.0% acid to phenolphthalein.

(7) Add 1.0% of any desired carbohydrate, alcohol, etc.

(8) Sterilize in the autoclave or flowing steam.

References: Percival (1920 p. 308), Harvey (1921–22 pp. 68, 108).

756. Rogers, Clark and Evans' Basal Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. Beef extract	4.0 g.
4. Na_2HPO_4	5.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 1.0% of one of the added nutrients in 1.

Sterilization: Not specified.

Use: To determine fermentation of carbohydrates, alcohols, etc. by bacteria.

Added nutrients and variants:

(a) The authors added 1.0% of one of the following materials to the basic solution:

glucose
sucrose
lactose
raffinose
starch
inulin
mannitol
glycerol
adonitol
dulcitol

(b) Rogers, Clark and Davis used 5.0 g. K_2HPO_4 instead of 5.0 g. Na_2HPO_4. They added 1.0% of one of the following materials:

glucose
levulose
galactose
adonitol
sucrose
lactose
dulcitol
raffinose
starch
inulin
mannitol
glycerol
salicin

(c) Evans used 5.0 g. K_2HPO_4 instead of 5.0 g. Na_2HPO_4 and added 1.0% of any desired carbohydrate, alcohol, etc.

(d) Sherman and Albus used 3.0 g. beef extract instead of 4.0 g. and 5.0 g. K_2HPO_4 instead of 5.0 g. Na_2HPO_4. They added 1.0% of one of the following test materials:

glucose
galactose
levulose
maltose
lactose
sucrose
raffinose
inulin
starch
glycerol
mannitol
salicin
dextrin

They omitted the K_2HPO_4 when glucose, galactose, levulose and maltose were added.

References: Rogers, Clark and Evans (1914 p. 111), Rogers, Clark and Davis (1914 p. 421), Evans (1916 p. 445), Sherman and Albus (1918 p. 162).

757. Stern's Basal Fuchsin Sulphite Broth

Constituents:

1. Water	1000.0 cc.
2. Peptone	20.0 g.
3. Meat extract	10.0 g.
4. NaCl	5.0 g.
5. Fuchsin (10.0% alcoholic solution)	
6. Na_2SO_4 (10.0% water solution)	
7. Chrysoidine (0.25% solution)	

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Adjust the reaction so that it is basic to phenolphthalein.
(3) Boil and filter.
(4) Prepare a saturated alcoholic fuchsin solution. Add about 10.0 g. fuchsin to 100.0 g. alcohol and allow to stand in the thermostat for 24 hours. Filter and place in dropping glass.
(5) Prepare a fresh 10.0% watery sodium sulfite solution.
(6) Prepare a 0.025% watery chrysoidine solution.
(7) To each 100.0 cc. of sterile (3) add 5 to 6 drops alcoholic fuchsin, 2.0 cc. sodium sulfite solution, 0.5 to 1.0 cc. chrysoidine solution and one of the added nutrients.
(8) Distribute into tubes.

Sterilization: Sterilize (3); method not given. Sterilize the tubed medium; method not given.

Use: Differentiation of the colon-typhoid group.

Added nutrients: The author added 1.0% of one of the following:

glucose
levulose
galactose
maltose
sucrose
xylose
arabinose
mannitol
glycerol

Reference: Stern (1916 p. 483).

758. Winslow, Rothberg and Parsons Basal Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Beef extract (Bacto)	3.0 g.
3. Peptone (Bacto)	5.0 g.
4. Brom cresol purple (0.04% alcoholic solution	27.0 cc.
5. Carbohydrate	5.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Add 27.0 cc. of a 0.04% alcoholic solution of brom cresol purple.
(3) pH = 6.7 to 6.8.
(4) Add 0.5% of one of the nutrients to sterile (3).

Sterilization: Sterilize at 15 pounds for 15 minutes.

Use: To study fermentation of carbohydrates, alcohols, etc., by bacteria. Author used streptococci.

Added nutrients: The author added 0.5% of any desired carbohydrate, alcohol, etc.

Reference: Winslow, Rothberg and Parsons (1923 p. 151).

759. Elser and Huntoon's Basal Nährstoff Heyden Broth

Constituents:

1. Distilled water	1000.0 cc.

2. Peptone (Witte)	10.0 g.
3. Nährstoff Heyden	10.0 g.
4. NaCl	5.0 g.
5. Litmus solution (Merck)	5.0 to 7.0 cc.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add 5.0 to 7.5 cc. of a watery solution of Merck's highly sensitized litmus.

(3) Prepare 100.0 cc. of a 10.0% solution of one of the added nutrients in distilled water.

(4) Mix sterile (2) and sterile (3).

(5) Tube.

(6) Incubate for 3 days to detect accidental contamination.

Sterilization: Sterilize (2) in the usual manner (method not given). Sterilize (3) at 100°C. for 10 minutes.

Use: To study fermentation of carbohydrates, alcohols, etc. by bacteria. The author used meningococci.

Added nutrients: The authors added 1.0% of one of the following:

glucose
sucrose
galactose
levulose
lactose
maltose
mannitol
dulcitol
inulin
dextrin

Reference: Elser and Huntoon (1909 p. 404).

760. Stutzer's Basal Nitrate Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Meat extract	5.0 g.
3. Peptone	5.0 g.
4. K_2HPO_4	1.0 g.
5. KNO_3	2.5 to 3.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and one of the added nutrients in 1.

Sterilization: Method not given.

Use: To study denitrification. Author reported that when glucose is used as a carbon source, no decomposition of KNO_3 takes place. If the salt of an organic acid (lactic acid) is used as a carbon source the KNO_3 is decomposed.

Added nutrients: The author added 10.0 g. of glucose, or sodium lactate.

Reference: Stutzer (1901 p. 82).

761. Bacto (Dehydrated) Basal Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Beef extract (Bacto)	3.0 g.
3. Peptone (Bacto)	5.0 g.

Preparation:

(1) These basic media are prepared in dehydrated form and contain 5 parts (0.5%) of one of the added nutrients. Dissolve 13.0 g. of the dehydrated medium in 1000.0 cc. of distilled water.

(2) pH = 6.6 + if sterilized for 20 minutes at 15 pounds pressure.

Sterilization: Sterilize in the usual manner.

Use: General culture medium and with the addition of carbohydrates to study fermentation. This medium conforms to the "Standard Methods 1920" formula.

Added nutrients:

(a) Digestive Ferments Co. prepare dehydrated glucose and lactose broth.

(b) Digestive Ferments Co. prepare an Andrade Basic Medium to which they add 0.5% sucrose, lactose, mannitol, maltose and glucose. This dehydrated medium is the same as the one given above except that 0.025 g. (0.0025%) of Difco Andrade Indicator is added.

Reference: Digestive Ferments Co. (1925 pp. 10, 15, 16).

762. Thoinot and Masselin's Basal Bouillon

Constituents:

1. Bouillon	1000.0 cc.

Preparation: (1) Add one of the nutrients to a liter of sterile peptone bouillon.

Sterilization: Method not given.

Use: To study fermentation of carbohydrates, alcohols, etc. by bacteria.

Added nutrients and variants:

(a) The authors added from 3.0 to 4.0% of glucose, lactose or glycerol to the basic solution.

(b) Roux and Rochaix added 1.0 to 2.0% glucose, 2.0% lactose or 1.0 to 10.0% glycerol to peptone bouillon. They then added 10.0 cc. of a 5.0% neutral red solution or sufficient litmus to give a color.

(c) Douglas, Fleming and Colebrook added a piece of one of the following to bouillon.

potato
bran
carrot
cabbage
charcoal
cork
sand
card

grape
asbestos wool
cotton wool
lint
sponge
blotting paper
khaki cloth
rusty nail
capillary tube

They studied the cultivation of wound anaerobes, *B. perfringens Bacillus malignant oedema.* The authors reported that the tube containing the blotting paper showed no growth and that growth took place first in the interstices of the porous material. Anaerobic conditions were probably produced in these places.

(d) Roddy added 0.5 to 2.0% of any desired carbohydrate, alcohol, etc., to bouillon. (Added 6.0% glycerol.)
(e) Dopter and Sacquépée added 2.0% of any desired carbohydrate, alcohol, etc., to a liter of sugar free peptone bouillon.

References: Thoinot and Masselin (1902 p. 24), Roux and Rochaix (1911 p. 109), Douglas, Fleming and Colebrook (1917 p. 530), Roddy (1917 p. 41), Dopter and Sacquépée (1921 p. 118).

763. Klecki's Basal Bouillon

Constituents:
1. Bouillon.................... 1000.0 cc.
2. Peptone.................... 10.0 g.
3. NaCl.................... 5.0 g.

Preparation:
(1) Preparation or composition of bouillon not given.
(2) Dissolve 2, 3 and one of the added nutrients in (1).
(3) Distribute in Pasteur fermentation tubes.

Sterilization: Method not given.

Use: To study fermentation by *Bacillus saccharobutyricus.* Author reported that butyric acid odor was present. Fermentation lasts much longer in the lactose bouillon than in the lactate medium. Fermentation starts after 13 to 16 hours.

Added nutrients: The author added 100.0 g. of lactose or 100.0 g. of calcium lactate.

Reference: Klecki (1896 p. 254).

764. Tanner's Basal Carbonate Bouillon

Constituents:
(1) Bouillon.................... 1000.0 cc.
(2) $CaCO_3$ (2.0%).............. 20.0 g.

Preparation:
(1) Dissolve one of the added nutrients in peptone bouillon.
(2) Add 2.0% of $CaCO_3$ to (1).

Sterilization: Method not given.

Use: To study fermentation of carbohydrates, alcohols, etc., by bacteria.

Variants: Dopter and Sacquépée added a small piece of $CaCO_3$ to each tube.

Added nutrients:
(a) The author added 1.0% of any desired carbohydrate, alcohol, etc., to the basic solution.
(b) Dopter and Sacquépée added 2.0% of any desired carbohydrate, alcohol, etc., to the basic solution.

References: Tanner (1919 p. 47), Dopter and Sacquépée (1921 p. 119).

765. Wolf's Basal Casein Digest Infusion Broth (Kahn)

Constituents:
1. Water.................... 1000.0 cc.
2. Beef infusion
3. Casein.................... 200.0 g.

Preparation:
(1) Add 20.0 g. anhydrous Na_2CO_3 to 1 liter of tap water in a 2 liter flask.
(2) Boil, and place on a water bath.
(3) Add 200.0 g. casein by dusting it in gradually, shaking from time to time to avoid lumps.
(4) Allow the mixture to cool and add 3.0 g. pancreatin and 15.0 cc. chloroform.
(5) Incubate for 5 days at 38°C. shaking vigorously each day to break up any crusts that form.
(6) Add 3.0 g. more of pancreatin and incubate for 10 days longer.
(7) At the end of the 15 days, add 400.0 cc. N/1 HCl.
(8) Steam for 30 minutes and filter.
(9) Add 120.0 cc. N/1 NaOH and adjust the reaction to pH = 7.2.
(10) Prepare beef infusion broth in the usual manner (details not given).
(11) Adjust (10) to pH = 7.0.
(12) Add 2.0% of (9) to (11).
(13) Seed with a strain of *B. coli* which actively ferments dextrose.
(14) Incubate for 24 hours.
(15) Heat in the sterilizer for 45 minutes and adjust to pH 7.2 by adding N/1 NaOH.

(16) Heat in the Arnold for 15 minutes and filter.
(17) Tube in 9.0 cc. quantities.
(18) Prepare 10.0% solutions of one of the added nutrients in the sugar free broth (16).
(19) Add 1.0 cc. of sterile (18) to each sterile tube of (17).
(20) Cap the tubes with a vaseline cap.
(21) Heat in the Arnold for 10 minutes.

Sterilization: Sterilize (17) in the autoclave. Sterilize (18) by heating in the Arnold at 100°C. for 10 minutes.

Use: To study the fermentation of carbohydrates, alcohols, etc., by bacteria. Kahn used the spore bearing anaerobes. Inoculation was made thru the liquid vaseline by means of a Pasteur pipette, and immediately immersing the tubes in cold water.

Added nutrients: The author added 1.0% of one of the following:

glucose	levulose
galactose	maltose
sucrose	lactose
raffinose	mannose
xylose	arabinose
starch	inulin
dextrin	salicin
mannitol	melezitose
glycerol	inositol

Reference: Kahn (1922 p. 166).

766. Torrey and Buckell's Basal Ascitic Fluid Infusion Broth

Constituents:

1. Beef infusion	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.
4. Ascitic fluid	200.0 cc.

Preparation:

(1) Method of preparation or exact composition of beef infusion not given.
(2) Inoculate (1) with *B. coli* and incubate 24 hours. Filter.
(3) To filtrate add 2 and 3 and dissolve.
(4) Adjust reaction to pH = 7.0.
(5) Tube in 5.0 cc. lots.
(6) To each tube of sterile (5) add 1.0 cc. sterile ascitic fluid. The fluid stored in the ice box for a long period to hydrolyze the fermentable materials that it might contain.
(7) Prepare 12.0% solutions of one of the added nutrients.
(8) Add 0.5 cc. of sterile (7) to each tube of (6).
(9) Incubate 3 days at 37°C. to determine sterility.

Sterilization: Sterilize (5) in the autoclave. Sterilize (8) by heating in flowing steam at 100°C. for 12 minutes.

Use: To study fermentation of carbohydrates and alcohols by bacteria. Author used gonococci.

Added nutrients: The authors added 12.0 g. of one of the following materials:

glucose	maltose
galactose	levulose

Reference: Torrey and Buckell (1922 p. 142).

767. Symmers and Wilson's Basal Ascitic Fluid Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Meat extract (Lemco)	1.0 g.
3. $NaHCO_3$	1.0 g.
4. Peptone	1.0 g.
5. Litmus (10.0% soln.)	100.0 cc.
6. Ascitic fluid	100.0 cc.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Dissolve 1.0% of one of the added nutrients to sterile (1).
(3) Add 10.0 cc. of sterile ascitic fluid to each 100.0 cc. of sterile (2).
(4) Tube in sterile tubes.
(5) Incubate at 37°C. to test sterility.

Sterilization: Sterilize (1) in the autoclave. Sterilize (2) by steaming on each of 3 successive days for 10 minutes. When using levulose, arabinose and xylose heat over the free flame instead of the steamer.

Use: To study fermentation of carbohydrates, alcohols, etc., by bacteria. Authors used meningococci.

Added nutrients and variants:

(a) The authors added 1.0% of any desired carbohydrate, alcohol, etc., to the basic solution.
(b) Rosenow and Towne obtained initial growth of the causative agent of poliomyelitis, pleomorphic streptococci, using a medium prepared as follows:

(1) Prepare an extract broth using Liebig's extract and peptone.
(2) Titrate and adjust reaction of (1) to 0.6–0.8 acid to phenolphthalein.
(3) Add 2.0% glucose, if desired, and 10.0% ascitic fluid.
(4) Distribute in six ounce nursing bottles containing 150.0 cc. of fluid in a column 4.5 cc. wide and 8.75 cc. high.
(5) Fresh sterile pieces of rabbit kidney may be added, if desired.
(6) Method of sterilization not given.

References: Symmers and Wilson (1909 p. 9), Rosenow and Towne (1917 p. 176).

768. Holman's Basal Serum Extract Broth

Constituents:

1. Distilled water	500.0 cc.
2. Beef serum	100.0 cc.
3. Peptone (Witte)	4.0 g.
4. Meat extract (Liebig's)	1.2 g.
5. NaCl	2.0 g.
6. Andrade's Indicator	4.0 cc.

Preparation:

(1) Collect beef blood from abattoir in sterile quart jars.
(2) Allow blood to clot in a cool room of the abattoir for about 15 minutes.
(3) Take to laboratory and allow to stand over night in a cool place.
(4) Obtain clear serum by centrifugation.
(5) Add 100.0 cc. of serum to 300.0 cc. distilled water.
(6) Dissolve 3, 4 and 5 in 200.0 cc. of distilled water.
(7) Adjust to +1.2% acid.
(8) Add 4.0 cc. of Andrade's indicator and 4.0 g. of one of the nutrients listed below.
(9) To cool sterile (8) add 500.0 cc. of sterile (5).
(10) Tube by means of a sterile tubing funnel or by means of a sterile syphon.
(11) Incubate several days to insure sterility.

Sterilization: Sterilize (5) by filtering thru a Berkefeld filter. Sterilize (8) in flowing steam 15 to 20 minutes on each of 3 successive days.

Use: To study fermentation of carbohydrates, alcohols, etc. by bacteria. The author used streptococci.

Added nutrients and variants:

(a) The author added 4.0 g. of any desired carbohydrate, alcohol, etc.
(b) The author prepared a similar medium in the following manner:
  (1) Collect beef blood from abattoir in sterile quart jars.
  (2) Allow blood to clot in the cool room of the abattoir for about 15 minutes.
  (3) Take to laboratory and allow to stand over night in a cool place.
  (4) Obtain clear serum by centrifugation.
  (5) Add 100.0 cc. of serum to 300.0 cc. distilled water.
  (6) Sterilize for 15 to 20 minutes in flowing steam on 3 successive days.
  (7) Dissolve 40.0 g. Witte's peptone, 12.0 g. Liebig's meat extract and 20.0 g. NaCl in 1000.0 cc. distilled water.
  (8) Titrate while hot to neutral to phenolphthalein.
  (9) Add 4.0 g. of any desired carbohydrate and 4.0 cc. of Andrade's indicator per 100.0 cc.
  (10) Sterilize in flowing steam for 15 to 20 minutes on 3 successive days.
  (11) The remaining method of preparation is somewhat ambiguous.

"The sterile serum water was then mixed with this quadruple strength carbohydrate broth, and the medium, consisting of 1 part serum to 4 parts 1% carbohydrate broth, was tubed into sterile tubes by means of a sterile tubing-funnel, or by use of a sterile syphon."

Reference: Holman (1914 pp. 210, 211).

769. MacConkey's Basal Bile Salt Iodide Bouillon

Constituents:

1. Nutrient bouillon	100.0 g.
2. Sodium taurocholate (Commercial)	0.5 g.
3. KI	0.5 g.

Preparation:

(1) Preparation of nutrient bouillon not given.
(2) Dissolve 2, 3 and one of the added nutrients in (1).
(3) Tube in fermentation tubes.

Sterilization: Sterilize in a steamer on 2 successive days for 10 minutes, taking care not to overheat.

Use: Enrichment of lactose fermenters. The author reported that the addition of KI may tend to delay the anaerobic growth while not interfere with aerobic multiplication.

Added nutrients: The author added 0.5 g. glucose, 1.0 g. sucrose or 0.5 g. lactose.

Reference: MacConkey (1905 p. 338).

770. Klimenko's Basal Glycerol Bouillon

Constituents:

1. Peptone bouillon...........	1000.0 cc.
2. Glycerol (1.0%)............	10.0 g.

Preparation:

(1) Preparation of peptone bouillon not given.

(2) Add (1.0%) glycerol to (1).

(3) Mix equal parts of (2) and one of the added nutrients.

Sterilization: Not specified.

Use: Cultivation of whooping cough bacillus. Author reported that the organism grows first on the surface and then on the bottom of the tube.

Added nutrients: The author mixed equal parts of one of the following and the basic solution:

ascitic fluid
blood serum
defibrinated blood

Reference: Klimenko (1909 p. 312).

771. Cantani's Basal Ascitic Fluid Bouillon

Constituents:

1. Bouillon
2. Ascitic fluid
3. Glycerol

Preparation:

(1) Mix equal parts of glycerol and one of the added nutrients. Storing the material with the glycerol tends to sterilize the material.

(2) After some time test the sterility of (1).

(3) Exact composition of bouillon not given.

(4) Tube (3).

(5) Add 6 parts of sterile ascitic fluid to 1 part (2).

(6) Add 0.5 to 0.75 cc. of (5) to each sterile tube of (4).

Sterilization: Method not specified.

Use: Cultivation of highly parasitic and pathogenic bacteria.

Added nutrients and variants:

(a) The author mixed glycerol with equal parts of urine, pus, milk, egg white or other albuminous fluids.

(b) The author used a mixture of equal parts of ascitic fluid and glycerol instead of ascitic fluid in step (5) above.

Reference: Cantani (1910 p. 472).

772. Buerger's Basal Ascitic Fluid Bouillon

Constituents:

1. Bouillon sugar free.........	1000.0 cc.
2. Ascitic fluid................	333.0 cc.
3. Litmus	

Preparation:

(1) Preparation of sugar free bouillon not given.

(2) Add litmus solution to (1) until a desirable color is obtained.

(3) Add $\frac{1}{3}$ volume of sterile ascitic fluid to sterile (2).

(4) Add sterile watery concentrated solutions of one of the added nutrients to (3) until a 1.0% strength is obtained.

(5) Tube into sterile tubes.

(6) Incubate in incubator to test sterility.

Sterilization: Method of sterilization of (2) not given. Sterilize the solution of added nutrients by heating on each of 3 successive days.

Use: To determine the fermentation of carbohydrates, alcohols, etc., by bacteria. Buerger used streptococci.

Added nutrients and variants:

(a) The author added 1.0% of one of the following materials:

arabinose
rhamnose
glucose
levulose
galactose
mannitol
dulcitol
sucrose
lactose
maltos
dextrin
inulin

(b) Elser and Huntoon prepared a similar medium using one part ascitic fluid with two parts bouillon. The procedure was as follows:

(1) Mix two parts sterile broth (exact method of preparation or composition not given) with one part sterile ascitic fluid. Method of sterilization not specified.

(2) Add 3.0 to 4.5 cc. of a watery solution of Merck's highly sensitized litmus.
(3) Prepare a 10.0% solution of one of the following in distilled water: glucose, galactose, levulose, lactose, maltose, sucrose, mannitol, dulcitol, inulin and dextrin.
(4) Sterilize (3) at 100°C. for 10 minutes.
(5) Mix (2) and (4).
(6) Tube in sterile tubes.
(7) Incubate for 3 days to detect accidental contamination.

Fermentation by the meningococci was studied in these media.

References: Buerger (1907 p. 430), Elser and Huntoon (1909 p. 404).

773. Akatsu's Basal Ascitic Fluid Bouillon

Constituents:
1. Bouillon.................... 1000.0 cc.
2. Ascitic fluid................ 1000.0 cc.
3. Tissue

Preparation:
(1) Composition or method of preparation of bouillon not given.
(2) Mix equal parts of bouillon and ascitic fluid and tube in 10.0 cc. lots.
(3) Add to each tube of (2) a piece of fresh tissue.
(4) Add 1.0% of one of the added nutrients to each tube.

Sterilization: Not specified.

Use: To study the fermentation of carbohydrates, alcohols, etc., by bacteria. Akatsu used spirochetes, *Treponema pallidum*, *Treponema calligyrum*, *Treponema microdentium*, *Treponema mucosum* and *Spirochaeta refringens*. Medium was covered with a layer of sterile paraffin after inoculation and incubated at 36°C.

Added nutrients: The author added 1.0% of one of the following materials:

amygdalin
arabinose
beerwort
dextrin
mannitol
raffinose
sucrose
starch
galactose
glycogen
glucose
inulin
lactose
levulose
maltose

Reference: Akatsu (1917 p. 376).

774. Hiss' Basal Serum Bouillon

Constituents:
1. Bouillon (sugar free)....... 1000.0 cc.
2. Serum (beef)............... 500.0 cc.

Preparation:
(1) Method of preparation of sugar free broth not given.
(2) Adjust (1) to 1.0% acid to phenolphthalein (neutral to litmus).
(3) Mix 1 part sterile beef serum with 2 parts sterile (2).
(4) Dissolve one of the added nutrients in (3).

Sterilization: Sterilize at 65–68°C. for one hour on 6 consecutive days.

Use: Differentiation of streptococci and pneumococci. Pneumococci fermented starch. Streptococci did not. Streptococci and pneumococci fermented lactose, saccharose and dextrin, produced acid and formed a yellowish white coagulum.

Added nutrients and variants:
(a) The author added 1.0% sucrose, 1.0% lactose, 1.0% glucose, 1.0% dextrin or 0.66% starch.
(b) Koch and Pokschischewsky prepared similar media for the differentiation of *Streptococcus longus seu. erysipelatos* and *Streptococcus equi*. They reported that *Streptococcus equi* gave a flocculent flaky sediment leaving a clear bouillon. *Streptococcus longus seu erysipelatos* gave a uniform turbidity to the medium. (Medium containing no sugars). The media were prepared as follows:
  (1) Mix one part sterile horse serum and two parts ordinary bouillon.
  (2) Sterilize on 3 successive days at 60°C. for one hour.
  (3) Prepare a 10.0% solution of one of the following:

sorbitol
dulcitol
mannitol
levulose
galactose
sucrose
maltose
lactose
glucose
mannose
raffinose

  (4) Sterilize on 3 successive days for 2 or 3 minutes in streaming steam.
  (5) Add 1.0 cc. of (4) to 9.0 cc. of (2), under aseptic conditions.

The medium was also employed without the addition of carbohydrates, alcohols, etc.

(c) Klimmer added 0.5 to 1.0% of any desired carbohydrate, alcohol, etc., to bouillon, and mixed one part of serum with one to twenty parts of the sugar bouillon.

References: Hiss (1901–05 p. 323), Koch and Pokschischewsky (1913 p. 10), Klimmer (1923 p. 200).

775. Holman's Basal Serum Bouillon

Constituents:

1. Water	400.0 cc.
2. Bouillon, double strength	400.0 cc.
3. Andrade's Indicator	80.0 cc.
4. Beef serum	200.0 cc.

Preparation:

(1) Preparation of double strength bouillon not given.
(2) Adjust (1) to +1.2.
(3) Add 200.0 cc. of water to 400.0 cc. of (1).
(4) Dissolve 8.0 g. of one of the added nutrients in 80.0 cc. of Andrade's indicator and add to (3).
(5) Add 200.0 cc. of water to 200.0 cc. of beef serum.
(6) Mix 400.0 cc. of sterile (5) and 600.0 cc. sterile (3).
(7) Tube into sterile test tubes.
(8) Incubate 2 days to determine sterility.

Sterilization: Sterilize (4) by heating in flowing steam on each of 3 successive days. Sterilize (5) by filtering thru a Berkefeld filter.

Use: To study fermentation of carbohydrates, alcohols, etc., by bacteria. The author used streptococci. The production of acid coagulates the serum.

Added nutrients and variants: The author added 8.0 g. of any desired carbohydrate, alcohol, etc. to the basic solution.

References: Holman (1916 p. 385), Stitt (1923 p. 36).

776. Ragit's Dehydrated Broth

Constituents:

1. Water	1000.0 cc.
2. Ragit Bouillon (Marck)	22.0 g.

Preparation: (1) Dissolve 2 in 1.

Sterilization: Not specified.

Use: This solution is equivalent to a 1.0% peptone broth.

Variants: Löhnis prepared the medium as follows:

(1) Heat 22.0 g. of Ragit bouillon in a liter of tap water in the steam sterilizer for an hour.
(2) Filter thru filter paper.
(3) Add concentrated soda solution until red litmus paper is turned slightly blue.
(4) Heat for 30 to 45 minutes in the steam sterilizer.
(5) Filter.
(6) Sterilization not specified.

References: Marx (1910 p. 361), Löhnis (1913 p. 14).

777. Bacto Dehydrated Broth

Constituents:

1. Distilled water	1000.0 cc.
2. Bacto bouillon (0.8%)	8.0 g.

Preparation:

(1) Dissolve 2 in 1. (Prepared according to directions given on the bottle).
(2) Initial pH = 7.1. (If sterilized at 15 pounds pressure for 20 minutes pH = 6.5+.)

Sterilization: Sterilize in the usual manner.

Use: General culture medium.

References: Brown (1921 p. 562), Digestive Ferments Co. (1925 p. 10).

778. Harvey and Iyengar's Dehydrated Broth

Constituents: 1. Trypsinized mutton infusion broth.

Preparation:

(1) Preparation of tryptic digest of mutton infusion broth not given.
(2) Adjust (1) to pH = 8.0.
(3) Cut up agar fibre into small pieces.
(4) Add (3) to (2) (6.0% by weight).
(5) Autoclave at 120° for an hour to melt the agar.
(6) Filter thru cotton, wool and muslin into a tin receptacle.
(7) Cut the agar out of the receptacle and into slices.
(8) Pass slices thru meat mincing machine with a finely perforated outlet disc.
(9) Spread minced nutrient agar on metal or other type of trays.

(10) Dry in hot air oven or any other convenient way.
(11) Store powder in sterile glass stoppered bottle.
(12) Add 6.0% by weight (11) to cold water.
(13) Extract (12) at room temperature for 2 hours.
(14) Filter.

Sterilization: Method of sterilization of the filtrate not given.

Use: General culture medium.

Reference: Harvey and Iyengar (1921–22 p. 366).

779. Dunham's Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.
3. NaCl (0.5%)	5.0 g.
4. Peptone (1.0%)	10.0 g.

Preparation:

(1) Boil finely chopped beef with a double weight of water for 2 hours.
(2) Filter.
(3) Make slightly alkaline.
(4) Dissolve 0.5% NaCl, and 1.0% peptone in (3).

Sterilization: Not specified.

Use: Detection of cholera vibrio. Author reported that cholera vibrio gave a red ring where the H_2SO_4 and medium met, when concentrated H_2SO_4 was poured down the side of a culture tube. The medium is used as a general culture medium.

Variants: Numerous authors have prepared media from these constituents. The method of preparation varied in a number of respects as indicated below.

(a) Schultz (1891).
  (1) Place 500.0 g. of the best quality meat, without fat or tendons, in a glass container fitted with a lid.
  (2) Pour 1300.0 cc. of distilled water over the meat.
  (3) Store in a cool place until the next day.
  (4) Filter thru 4 thicknesses of "Morly" and press the remaining meat to obtain as much fluid as possible.
  (5) Pour the filtrate into a kettle, and add 10.0 g. peptone (siccum), 5.0 g. NaCl and the whites of two eggs, beaten up in 2 or 3 volumes of water.
  (6) Boil under a gas flame for 15 minutes.
  (7) Adjust to faint alkalinity, using phenolphthalein as an indicator, and the end point being a faint red color.
  (8) Pour into an iron kettle, add 100.0 cc. distilled water, boil strongly for 5 minutes and filter.
  (9) Distribute.
  (10) Sterilization not specified.

(b) Frothingham (1895).
  (1) Add one pound of finely chopped lean meat to 1000.0 cc. of water and allow to stand from 12 to 24 hours in a cool place.
  (2) Strain thru a cheese cloth or a coarse towel and squeeze in a meat press or by twisting the ends of the cloth until 1000.0 cc. of the meat juice is obtained. Make up to 1000.0 cc. by the addition of water if necessary.
  (3) Dissolve 5.0 g. NaCl and 10.0 g. dried peptone in (2).
  (4) Boil 15 minutes, either in steam or over a flame.
  (5) Make the reaction slightly alkaline by the addition of a saturated solution of Na_2CO_3.
  (6) Boil from 45 minutes to an hour.
  (7) Filter. The filtrate should be clear. If not, add the yolks of 2 eggs, mix rapidly and boil for a quarter to a half hour. Filter.
  (8) Sterilization not specified.

(c) Nicolle (1896).
  (1) Infuse 500.0 g. of fresh finely chopped beef with 1 liter of water overnight at 10°C. to 12°C.
  (2) Add 2.0% peptone and 0.5% NaCl to (1).
  (3) Boil, filter and make rather strongly alkaline.
  (4) Heat for 10 minutes at 120°C.
  (5) Filter and distribute in flasks in the ratio of one liter medium to a two liter flask.
  (6) Sterilize for 15 minutes at 115°C.

(d) Stutzer and Hartleb (1897).

(1) Add 2 liters of water to 1000.0 g. of finely chopped lean meat.
(2) Heat in the steamer for 2 hours.
(3) Pour off the liquid.
(4) Make (3) up to 3 liters.
(5) Neutralize by the addition of soda, indicator not specified.
(6) Filter.
(7) Sterilize (Method not given).
(8) Dissolve 2.0% peptone, 10.0 g. NaCl, and 10.0 cc. of a 10.0% Na_2CO_3 solution in (7).

(e) Wurtz (1897).
(1) Chop 500.0 g. of lean beef (or other meat) into fine pieces.
(2) Add a liter of water and place in the cold for 24 hours.
(3) Pass thru a sieve.
(4) Filter thru a linen towel and free the meat from juice.
(5) Add sufficient water to make the volume up to 1000.0 cc.
(6) Add 10.0 g. peptone and 5.0 g. NaCl.
(7) Heat in the autoclave at 125° for 15 minutes. (30 minutes for 2 liters.)
(8) Filter.
(9) Make slightly alkaline to litmus.
(10) Heat for 10 to 15 minutes at 125°C.
(11) Allow to stand for 12 hours.
(12) Filter.
(13) Distribute in sterile test tubes or Erlenmeyer flasks.
(14) Sterilize at 115° for 15 minutes.

(f) Committee A. P. H. A. (1899).
(1) Macerate one part finely chopped lean meat with 2 parts distilled water in the ice box for 18 to 24 hours, stirring occasionally.
(2) Strain, cold, thru a fine cloth.
(3) Add 1.0% peptone and 0.5% NaCl to the filtrate. Heat until solution is complete.
(4) Add NaOH until the reaction is slightly alkaline (practically neutral) to phenolphthalein.
(5) Heat on a water bath for 30 minutes and boil for 5 minutes over a free flame.
(6) Filter while hot thru paper or cotton and cloth.
(7) Add N/1 HCl to the filtrate to obtain the desired reaction (+1.5).
(8) If the medium is clear distribute in tubes or flasks. If not clear, clarify by adding the white of one egg to the agar cooled to 50 to 60°C. and then boiled vigorously. Filter.
(9) Sterilize either by fractional or continuous method.

(g) Migula (1901).
(1) Mix 500.0 g. of finely chopped lean beef with 1 liter of water and allow to stand in the ice box for 12 to 24 hours.
(2) Press the liquid thru a towel and make up the volume to 1 liter.
(3) Boil in the steam cooker for 30 minutes.
(4) The infusion may be boiled for an hour before removing the meat and then filtered thru paper. If the liquid is still red, boil again for 15 minutes.
(5) Filter when cold to remove the fat.
(6) Add 10.0 g. Witte's peptone and 5.0 g. NaCl.
(7) Neutralize by the addition of a concentrated solution of Na_2CO_3 until litmus is colored violet.
(8) Add the desired amount of soda. Generally 10.0 cc. of 15.0% soda solution is added per liter.
(9) Boil and filter.
(10) Distribute in tubes or flasks.
(11) Boil for one hour to sterilize.

(h) Thionot and Masselin (1902).
(1) Macerate 500.0 g. of finely chopped beef with a liter of water for about 12 hours.
(2) Express the juice from the meat.
(3) Boil the juice slowly for several minutes.
(4) Filter on a wetted filter paper.
(5) Add 10.0 g. peptone and 5.0 g. NaCl to the filtrate.
(6) Make slightly alkaline to litmus by the addition of soda.
(7) Heat in the autoclave for 15 minutes at 115°C.
(8) Filter at once.
(9) Distribute in tubes or flasks.
(10) Sterilize at 115°C. for 20 minutes.

(i) Smith (1902).

(1) Soak 500.0 g. of finely chopped fresh fat free beef in 1000.0 cc. of boiled water over night in the ice box.
(2) Strain thru a piece of cheese cloth. Compress the pulp to obtain a liter of fluid. Make up to a liter if necessary.
(3) Heat between 50° and 60°C. and add 10.0 g. peptone and 5.0 g. NaCl. Stir constantly until solution is complete.
(4) Boil with constant stirring.
(5) Filter thru paper.
(6) Reboil and filter again if the filtrate is turbid.
(7) Cool to room temperature and make the volume to one liter.
(8) Adjust the reaction.
(9) Boil and filter.
(10) Tube or flask.
(11) Sterilize by the fractional method in steam at 100°C. or in the autoclave at 120°C.

(j) Frost (1903).

(1) Remove all the fat and connective tissue from 500.0 g. of beef and mince (or use hamburg steak).
(2) Add 1 liter of distilled water to (1), shake thoroughly and set in the ice box for 12 to 24 hours.
(3) Squeeze thru a cloth and add enough distilled water to make 1 liter.
(4) Add 1.0% peptone (Witte) and 0.5% NaCl to (3).
(5) Weigh the solution and vessel.
(6) Heat to not above 60°C. until the ingredients are in solution.
(7) Restore the loss in weight by the addition of distilled water.
(8) Neutralize to phenolphthalein by the addition of normal NaOH.
(9) Boil for 5 minutes and restore the weight.
(10) Test the reaction and readjust if necessary.
(11) Add 0.5 to 1.5% of normal HCl.
(12) Heat until the precipitate appears flaky.
(13) Filter thru moistened filter paper.
(14) Tube.
(15) Sterilize for 20 minutes in a steam sterilizer on 3 consecutive days or in the autoclave at 120° for 20 minutes.

(k) Frost (1903).

(1) Remove all the fat and connective tissue from 500.0 g. of beef and mince or use hamburg steak.
(2) Add 1 liter of distilled water.
(3) Place in a vessel for cooking and cook for 30 minutes at about 70°C.
(4) Filter thru paper and make up to one liter.
(5) Add 1.0% peptone (Witte) and 0.5% NaCl to (3).

The remainder of the preparation is identical with variant (j) above, from step (5). These two bouillons may be inoculated with *B. coli* and incubated at 38°C. for several hours to render them sugar free.

(l) Committee A. P. H. A. (1905).

(1) Infuse 500.0 g. finely chopped lean meat with 1000.0 cc. water for 24 hours in refrigerator.
(2) Make up volume.
(3) Strain thru cotton flannel.
(4) Add 10.0 g. Witte's peptone.
(5) Dissolve by heating in water bath.
(6) Heat over steam bath 30 minutes.
(7) Restore volume.
(8) Adjust reaction to +1 with phenolphthalein.
(9) Boil 2 minutes over free flame, stir.
(10) Restore volume.
(11) Filter thru absorbent cotton and cotton flannel until clear.
(12) Tube in 10.0 cc. lots.
(13) Sterilize—autoclave or by intermittent method.

(m) Heinemann (1905).

(1) Add 500.0 g. lean minced beef, free from fat and tissue as possible, to 1000.0 cc. tap water.
(2) Place on ice for 24 hours.
(3) To prepare a sugar free broth add a pure culture of Bacillus coli to (2) and incubate at 37° for 24 hours.

(4) Weigh the saucepan and cook for about 30 minutes.
(5) Strain thru cheese cloth, and press all the liquid out in a meat press.
(6) Replace the water lost by evaporation.
(7) Dissolve 10.0 g. of Witte's peptone in (6).
(8) Adjust the reaction.
(9) Filter into flasks.
(10) Sterilize in the autoclave at 120° for 10 minutes.
(11) Tube (sterile tubes?).

(n) Committee A. P. H. A. (1909).
(1) Infuse 500.0 g. finely chopped lean meat with 1000.0 cc. distilled water for 24 hours in the ice box.
(2) Make up the loss due to evaporation.
(3) Strain thru cotton flannel.
(4) Add 1.0% peptone and heat on the water bath until solution is complete.
(5) Heat over boiling water or steam for 30 minutes.
(6) Restore the loss due to evaporation.
(7) Add normal NaOH until the reaction is +1.0%.
(8) Boil 2 minutes over a free flame. Stir constantly.
(9) Make up the loss due to evaporation.
(10) Filter thru absorbent cotton until clear.
(11) Retitrate and record the reaction.
(12) Tube in 10.0 cc. quantities.
(13) Sterilize (method not given).

(o) Löhnis (1913).
(1) Mix one pound of lean unsalted minced meat with one liter of water.
(2) Allow to stand in a cool place for 12 to 24 hours.
(3) Filter thru cloth, squeezing the meat free from juice until 1 liter of juice is obtained. Make up to a liter by the addition of water, if necessary.
(4) Add 1.0% Witte's peptone and 0.5% NaCl.
(5) Heat for 60 minutes in the steam sterilizer.
(6) Filter thru filter paper.
(7) Add concentrated soda solution until red litmus paper is turned slightly blue.
(8) Heat for 30 to 45 minutes in the steam sterilizer.
(9) Filter.
(10) Sterilization not specified.

(p) Sears (1916).
(1) Grind beef fine and obtain juice in 1 liter of water (soaking or standing not specified).
(2) Dissolve 10.0 g. Witte's peptone and 5.0 g. NaCl in (1).
(3) Adjust to neutral to phenolphthalein.
(4) Distribute in 2-500.0 cc. lots.
(5) Sterilize at 15 pounds pressure (time not specified).

(q) Roddy (1917).
(1) Mix 500.0 g. of minced lean beef or veal in 1000.0 cc. of water.
(2) Place in an ice box for 24 hours in a shallow dish.
(3) Skim off the fat.
(4) Filter thru linen.
(5) Express the juice contained in the meat.
(6) Boil for 2 hours.
(7) Filter thru linen.
(8) Boil again for one hour.
(9) Add water to make up the loss due to evaporation.
(10) Add 10.0 g. of peptone and 5.0 g. NaCl.
(11) Boil for 30 minutes.
(12) Make up the loss due to evaporation.
(13) Adjust the reaction.
(14) Filter while hot and again when cool.
(15) Sterilize in the autoclave.

(r) Roddy (1917).

Prepared as variant (q) above, step (1) thru (11).

(12) Inoculate with a culture of *Bacillus coli*.
(13) Incubate for 24 hours at 37°.
(14) Boil for 30 minutes.
(15) Filter while hot and again when cool.

(16) Adjust the reaction.
(17) Sterilize in the autoclave.

(s) McIntosh and Smart (1918).

(1) Mince 1.0 pound of fat free meat and macerate in 1000.0 cc. cold water over night. Or the mixture may be macerated at 40°C. for 30 minutes instead.
(2) Boil for 15 minutes.
(3) Filter thru muslin and then paper.
(4) Add 10.0 g. peptone and 5.0 g. NaCl.
(5) Make up to 1000.0 cc., dissolve and steam for 45 minutes.
(6) Measure 10.0 cc. of the broth into a porcelain evaporating dish, and add 25.0 cc. of distilled water.
(7) Prepare another dish in the same way as a control.
(8) Add 5 drops of a 0.5% alcoholic solution of thymolphthalein to one dish.
(9) Add N/10 NaOH to (8) until the color changes from the yellow to a bluish tint.
(10) Calculate and add the amount of normal NaOH that would be required to give the same reaction to the remainder of the broth.
(11) Boil and filter.
(12) Add 10.0 cc. of N/1 HCl per liter of filtrate. This gives a pH = 7.6.
(13) Tube or flask and sterilize in the autoclave for 20 minutes at 115°C.

(t) Bezançon (1920).

(1) Soak 500.0 g. of finely chopped lean beef (or other meat) in 1000.0 cc. of water for 12 hours.
(2) Collect the liquid from the meat and add 10.0 g. peptone and 5.0 g. NaCl.
(3) Autoclave for 10 minutes at 115°C.
(4) Filter thru a wetted filter.
(5) Make slightly alkaline to litmus by the addition of 10.0% soda solution.
(6) Autoclave for 15 minutes at 120°C.
(7) Filter.
(8) Distribute as desired
(9) Sterilize in the autoclave at 115° for 15 minutes.

(u) Percival (1920).

(1) Chop one pound of lean, fat free beef with a knife or mincing machine.
(2) Place (1) in a porcelain dish or glass beaker and add 1 liter of water.
(3) Allow to soak over night in a cool place.
(4) Strain thru muslin, and boil for one hour.
(5) Filter thru filter paper into a large flask.
(6) Make the volume up to one liter.
(7) Add 10.0 g. Witte's peptone and 5.0 g. NaCl to (6).
(8) Neutralize to phenolphthalein.
(9) Steam for 15 minutes.
(10) Neutralize again and adjust the reaction to +10.
(11) Filter into sterile flasks or test tubes.
(12) Sterilize on 3 successive days for 20 minutes each time.

(v) Giltner (1921).

(1) Add 500.0 cc. of tap water to 500.0 g. of finely chopped fresh lean beef in a 3.5 liter agate-ware pail.
(2) Mix thoroly and allow to stand in a cool place (refrigerator) for not more than 16 to 24 hours.
(3) Strain the infusion thru a cheese cloth, thoroly pressing out all the juice.
(4) Make up to 500.0 cc. by the addition of tap water if necessary.
(5) Place (4) in a sterile liter Erlenmeyer flask.
(6) Heat in the autoclave at 120°C. for 30 minutes.
(7) Pour the contents of the flask into an agate pail and add 500.0 cc. of tap water.
(8) Add 1.0% Witte's peptone and 0.5% NaCl.
(9) Thoroly mix 10.0 g. of egg albumin with 100.0 cc. of tap water and add to (8) or one well beaten egg white may be used.
(10) Heat in the autoclave at 120°C. for one hour.

(11) Adjust the Reaction to +1.5% by the addition of NaOH or HCl.
(12) Filter thru the same paper until the medium is bright and clear.
(13) Distribute as desired.
(14) Sterilize in the autoclave at 120°C. for 30 minutes.

(w) Dopter and Sacquépée (1921).
(1) Add 1000.0 cc. of water to 500.0 g. of finely chopped fat and tendon free beef.
(2) Allow to stand in the ice box for 12 hours, or heat at 50 to 55° for 30 minutes.
(3) Heat slowly to boiling.
(4) Boil slowly for 10 minutes stirring constantly.
(5) Press the liquid thru a clean cloth.
(6) Add 20.0 g. peptone and 5.0 g. NaCl 1000.0 g. of the filtrate and dissolve by shaking.
(7) Filter thru a wetted filter paper.
(8) Make slightly alkaline to litmus by the addition of NaOH or Na_2CO_3.
(9) Flask.
(10) Autoclave at 117° for 15 minutes.
(11) Filter thru filter paper.
(12) Make up to 1000.0 cc. by the addition of distilled water.
(13) Tube or flask.
(14) Sterilize at 115°C. for 20 minutes.

(x) Abbott (1921).
(1) Add 500.0 g. of chopped lean beef to 1 liter of water and soak for 24 hours, kept at ice box temperature.
(2) Strain thru a coarse towel and press until a liter of fluid is obtained.
(3) Add 10.0 g. (1.0%) dry peptone and 5.0 g. (0.5%) NaCl.
(4) Make slightly alkaline or neutral (indicator not specified).
(5) Place in a porcelain lined saucepan, and boil over a flame. Boil until all the albumin is coagulated and the fluid portion is of a clear pale straw color.
(6) Filter thru a folded paper.
(7) Sterilize by steam (method not given).

(y) Foster and Randall (1921).
(1) Prepare beef infusion broth from 1000.0 cc. water, 300.0 g. lean beef and 10.0 g. Parke Davis & Co. peptone (Procedure not given).
(2) Initial pH = 5.0 to 9.0, changing every 0.4 interval using N/10 or N/1 NaOH and N/10 or N/1 HCl.
(3) Sterilize at 15 pounds for 15 minutes.

(z) Randall and Hall (1921).
(1) Soak ground lean beef in water over night and then boil 10 minutes.
(2) Remove coagulated albumin by straining under pressure and paper filtration of the liquid.
(3) Add peptone (2.0%) and NaCl (0.5%).
(4) Adjust to any desired reaction.
(5) Method of sterilization not given.

(aa) Ayers, Rupp and Mudge (1921).
(1) Method of preparation or composition of infusion broth not given.
(2) Dissolve 40.0 g. Bacto peptone in (1).
(3) Adjust to pH = 7.5.
(4) Method of sterilization not given.

(bb) Harvey (1921–22).
(1) Mince finely fat-free beef.

NOTE: Veal, chicken, ox or horse heart, horse flesh, rabbit flesh, fish, blood, placenta, liver, spleen, kidneys, brain, and vegetable materials, such as yeast, wheat, etc., may serve to furnish the extract used as basis for the medium.

(2) Add 500.0 g. to 100.0 cc. distilled water or clear tap water.
(3) Heat the mixture 20 minutes over a free flame at a temperature not exceeding 50°C.

NOTE: Or simply keep in a cool place over night.

(4) Skim off fat floating on the surface.
(5) Raise the temperature to boiling point.
(6) Boil 10 minutes.
(7) Pour the mixture on to a wet, thick, clean cloth.

(8) Collect the fluid which drains thru the cloth together with that obtained by squeezing the meat in the cloth.
(9) Filter the fluid collected thru well-wetted, thick filter paper.
(10) Add to the filtrate: peptone 10.0 g., sodium chloride 5.0 g.

Note: The peptone should be worked into a paste or suspension by gradual addition of a little of the meat extract before addition to the filtrate.

(11) Steam or boil 45 minutes.
(12) Bring the volume up to 1000.0 cc. by the addition of water.
(13) Estimate and adjust reaction to a pH value or slightly alkaline to litmus or 1.0% acid to phenolphthalein.
(14) Steam 30 minutes.
(15) Clarify if necessary, and filter while hot thru well wetted, thick filter paper, or thru two layers of absorbent cotton wool.

Note: If simple filtration thru thick paper alone is not sufficient to give a clear medium, clearing should be effected by means of white of egg or other clearing agent.

(a) Beat up the white of one or two eggs along with the crushed shells in about 20.0 cc. water.

Note: Raw meat juice, 15.0 cc. per liter of medium may be substituted for white of egg.

(b) Add to the medium little by little before filtration and at a temperature not exceeding 60°C.
(c) Stir to mix.
(d) Steam for 30 minutes.
(e) Remove from steamer and shake up well to mix.
(f) Steam again 15 minutes.
(g) Filter in the steamer thru thick filter paper, or thru 2 layers of absorbent cotton wool.
(h) Refilter, if necessary, the first portion of the filtrate.

(16) Distribute the filtrate into flasks or test tubes.
(17) Sterilize.

(cc) Harvey (1921–22).

Prepare the medium like variant (aa) (1) thru (15).

(16) Inoculate bouillon with *B. coli*.
(17) Incubate 24 hours.
(18) Boil 20 minutes.
(19) Make a paste or suspension of about 15 g. purified talc with a little of the dead bouillon culture.
(20) Add the suspension to the culture.
(21) Filter and refilter thru filter paper till clear.
(22) Distribute into test tubes.
(23) Sterilize.

(dd) Harvey (1921–22).

(1) Mince finely fat-free beef.
(2) Add 500.0 g. to 1000.0 cc. distilled water or tap water.
(3) Keep in a cool place over night.
(4) Skim off fat floating on the surface.
(5) Pour the mixture on to a wet, thick, clean cloth.
(6) Collect the fluid which drains thru the cloth together with that obtained by squeezing the meat in the cloth.
(7) Bring the volume up to 1000.0 cc. by the addition of water.
(8) Add peptone 10.0 g., sodium chloride 5.0 g.
(9) Estimate and adjust the reaction to a definite pH or faintly alkaline to litmus or 1.0% acid to phenolphthalein.
(10) Steam or boil 45 minutes.
(11) Filter, while hot, thru well-wetted, thick filter paper, or thru 2 layers of absorbent cotton wool.
(12) Distribute the filtrate into flasks or test tubes.
(13) Sterilize (S9.5, 9.6 according to Harvey).

(ee) Heinemann (1922).

(1) Clean one pound of beef or veal of adhering fat, etc., and grind in a meat chopper.
(2) Cover with a liter of water.
(3) Digest over night at room temperature.
(4) Heat to 60°C. and digest at this temperature for two hours.

(5) Boil for 30 minutes.
(6) Press the liquid from the meat in a meat press.
(7) Mix the meat with more water, and press out again.
(8) Bring the volume of the combined liquids to 1 liter.
(9) Dissolve 20.0 g. peptone and 5.0 g. NaCl in (8).
(10) Adjust the reaction to 1.2% acid with phenolphthalein as an indicator.
(11) Filter until perfectly clear.
(12) Sterilize in the autoclave.

(ff) Pitfield (1922).
(1) Cover 500.0 g. of finely cut fat free beef with 1000.0 cc. of water.
(2) Shake well and place on ice over night.
(3) Squeeze out the fluid by means of a cloth and make up the volume to 1 liter.
(4) Inoculate with a culture of the colon bacillus.
(5) Allow to stand at room temperature over night.
(6) Boil and add 10.0 g. Witte's peptone and 5.0 g. NaCl.
(7) Weigh the sauce pan and contents and heat to 60°C.
(8) Make up the loss in weight by the addition of water.
(9) Neutralize to litmus.
(10) Boil 5 minutes.
(11) Make up the loss in weight by the addition of water.
(12) Adjust the reaction as desired (+0.5 to +1.5%).
(13) Filter.
(14) Distribute into flasks or tubes.
(15) Sterilize (method not given).

(gg) Hartley (1922) used the following medium for diphtheria toxin production:
(1) Soak 1 pound of lean finely chopped horse meat in 1000.0 cc. of tap water over night.
(2) Raise the temperature to 95°C. the following morning.
(3) Allow to cool slightly and filter thru paper pulp.
(4) Adjust the reaction of the filtrate to pH = 8.0.
(5) Dissolve 2.0% Park Davis bacteriological peptone and 0.5% NaCl in (4).
(6) Adjust to pH = 8.0.
(7) Distribute in desired containers.
(8) Pass steam thru the autoclave for one hour after which slowly raise the pressure to 10 pounds (time about 30 minutes) and keep at this temperature for 30 minutes.

(hh) Stitt (1923).
(1) Cut up 500.0 g. fat-free meat in a sausage mill.
(2) Pour 1000.0 cc. of water over (1).
(3) Keep in the ice chest over night.
(4) The following morning skim off the scum of fat by means of a piece of absorbent cotton.
(5) Squeeze out the infusion thru a strong muslin cloth, making the amount up to 1000.0 cc.
(6) Dissolve 1.0% Witte's peptone and 0.5% NaCl in (5). Mix the salt and peptone with a little of the infusion and prepare a paste of the mixture in a mortar before adding it to (5).
(7) Add sufficient normal NaOH to make the reaction +1.0 to phenolphthalein.
(8) Place in the inner chamber of a rice cooker and bring to boil. Boil for 20 minutes. It is necessary to have NaCl or $CaCl_2$ in the outer chamber. Do not stir the medium.
(9) Filter thru a wet filter paper.
(10) Make up the volume to 1000.0 cc. by the addition of distilled water.
(11) Readjust or record the reaction.
(12) Filter if the reaction is adjusted in (11).
(13) Sterilize in the autoclave at 115°C. for 15 minutes or in the Arnold on 3 successive days.

(ii) Stitt (1923).
Prepare the medium like the above variant (gg) (1) thru (12).
(13) Inoculate with 5.0 cc. of a 24-hour culture of the colon bacillus.
(14) Incubate over night at 37°C.
(15) Boil in a sauce pan.

- (16) Put 15.0 g. of purified talc (talcum purificatum U. S. P.) in a mortar.
- (17) Add (15) to (16), stirring constantly.
- (18) Filter thru paper until clear.
- (19) Adjust the reaction to +1.0 to phenolphthalein.
- (20) Sterilization not specified.

(jj) Klimmer (1923).

- (1) Preparation of meat infusion not specified.
- (2) Add 10.0 g. peptone and 5.0 g. NaCl to (1) and heat gently until solution is complete.
- (3) Boil.
- (4) Neutralize.
- (5) Neutralize to litmus and add 7.0 cc. normal soda solution per liter.
- (6) Filter.
- (7) Distribute as desired.
- (8) Sterilize in the autoclave with one heating or in streaming steam each on 3 successive days.

(kk) Cunningham (1924).

- (1) Mince or chop into as small pieces as possible 1 pound of lean unsalted meat, preferably rump steak or veal. The meat must be fat-free.
- (2) Cover (1) with a liter of water in a large beaker.
- (3) Allow to stand in a cool place for 24 hours.
- (4) Skim off the fat.
- (5) Strain thru a piece of cheese cloth.
- (6) Press out as much liquid as possible from the meat.
- (7) Make up to 1000.0 cc. by the addition of water.
- (8) Add 1.0% peptone and 0.5% NaCl.
- (9) Steam for an hour in a double walled pot (one hour from the time the water boils).
- (10) Add normal NaOH solution until the liquid turns a piece of moistened turmeric paper dipped into it faintly but distinctly brown. The reaction is acid to phenolphthalein, pH varies from 7.7 to 8.0.
- (11) Heat in an autoclave.
- (12) Filter thru grey coarse filter paper until clear.
- (13) Make up the volume to one liter with distilled water.
- (14) Tube in 8.0 cc. lots.
- (15) Sterilize at 22.5 pounds pressure.

(ll) Park, Williams and Krumwiede (1924).

- (1) Soak 5.0 pounds of finely chopped lean beef or veal in 5000.0 cc. tap water over night in an ice box or at room temperature.
- (2) Strain thru cheese cloth. Squeeze by twisting the cloth or use a meat press. This is meat juice.
- (3) Add 1.0% peptone and 1.0% NaCl to the filtrate.
- (4) Heat to 50°C. to dissolve peptone and NaCl.
- (5) Determine the reaction and adjust if necessary. (In this case boil again.)
- (6) Filter thru cotton and filter paper in a glass funnel.
- (7) "Sterilize in tubes or flasks by heating in an autoclave for an hour at 15 pounds pressure."

(mm) Park, Williams and Krumwiede (1924).

- (1) Soak 5.0 pounds of finely chopped lean beef or veal in 5000.0 cc. tap water over night in an ice box or at room temperature.
- (2) Weigh the kettle used in (1) and its contents.
- (3) Heat at 45°C. for an hour.
- (4) Boil for 30 minutes.
- (5) Make up the loss by weight by the addition of water.
- (6) Strain thru cheese cloth. Squeeze by twisting the cloth or use the meat press.
- (7) This may be sterilized in flasks and stored for future use. This is meat infusion.
- (8) If used at once, add 1.0% peptone and 0.5% NaCl.
- (9) Heat to boiling.
- (10) Determine the reaction and adjust if necessary (in this case boil again).

(11) Filter thru cotton and filter paper in a glass funnel.
(12) Sterilize in tubes or flasks by heating in an autoclave for an hour at 15 pounds pressure.

(nn) Kreidler (1926).
(1) Infuse 500.0 g. of finely chopped lean beef in 1000.0 cc. water in an ice box over night.
(2) Boil for 30 minutes over a free flame.
(3) Filter thru gauze and then thru paper to remove the fat.
(4) Make up to 1000.0 cc. volume.
(5) Add 10.0 g. peptone (Difco) and 5.0 g. NaCl.
(6) Boil to dissolve.
(7) Adjust to pH = 7.8.
(8) Filter thru paper.
(9) Sterilize in the Arnold using the fractional method.

References: Dunham (1887 p. 338), Schultz (1891 p. 56), Frothingham (1895 p. 53), Nicolle (1896 p. 333), Stutzer and Hartleb (1897 p. 404), Wurtz (1899 p. 26), Committee A. P. H. A. (1899 p. 77), Migula (1901 p. 19), Thionot and Masselin (1902 p. 19), Smith (1902 p. 80), Frost (1903 p. 6), Heinemann (1905 p. 27), Committee A. P. H. A. (1905 p. 107), (1909 p. 285), Smith (1905 p. 195), Löhnis (1913 p. 14), Sears (1916 p. 113), Roddy (1917 p. 41), McIntosh and Smart (1919 p. 724), Percival (1920 p. 56), Bezançon (1920 p. 108), Giltner (1921 p. 29), Dopter and Sacquépée (1921 p. 117), Abbott (1921 p. 124), Foster and Randall (1921 p. 151), Randall and Hall (1921 p. 347), Ayers, Rupp and Mudge (1921 p. 258), Harvey (1921–22 pp. 67, 68), Heinemann (1922 p. 31), Pitfield (1922 p. 13), Hartley and Hartley (1922 p. 460), Stitt (1923 p. 30), Klimmer (1923 p. 192), Frost (1923 p. 64), Cunningham (1924 p. 11), Park, Williams and Krumwiede (1924 p. 96), Kreidler (1926 p. 190).

780. Jensen's Nitrate Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Meat	500.0 g.
3. NaCl	5.0 g.
4. Peptone	10.0 g.
5. $NaNO_3$	3.0 g.

Preparation:
(1) Prepare a meat infusion from 1 and 2 (method not given).
(2) Dissolve 3, 4 and 5 in (1).
(3) Adjust to a slight alkalinity with soda.

Sterilization: Method not given.

Use: To study denitrification.

Variants:
(a) Percival prepared a similar medium by adding 3.0 g. $NaNO_3$ to a liter of meat infusion (See Dunham's Meat Infusion Peptone Solution, variant (u) 634).
(b) Harvey prepared a similar medium by adding 5.0 g. KNO_3 to a liter of meat infusion (see Dunham's Meat Infusion Peptone Solution, variant (bb) 634).

Reference: Jensen (1898 p. 406), Percival (1920 p. 56), Harvey (1921–22 p. 67).

781. Davis and Ferry's Nitrate Infusion Broth

Constituents:

1. Beef infusion solution	500.0 cc.
2. 2.0% peptone solution	500.0 cc.
3. NaCl	4.0 g.
4. K_2HPO_4	3.0 g.
5. $MgSO_4$	0.4 g.
6. KNO_3	0.2 g.

Preparation:
(1) Preparation of beef infusion not given.
(2) Mix 1 and 2.
(3) Dissolve 3, 4, 5 and 6 in (1).
(4) Add enough N/1 NaOH to give final reaction pH = 8.0 to 8.2.
(5) Steam 15 minutes and check reaction.
(6) Distribute as desired.

Sterilization: Sterilize at 115° for 20 minutes.

Use: Cultivation of *Bact. diphtheriae* and toxin production. Authors reported good growth.

Reference: Davis and Ferry (1919 p. 236).

782. Loeffler's Infusion Broth (Roux and Rochaix)

Constituents:

1. Distilled water	1000.0 cc.
2. Beef	500.0 g.
3. NaCl	5.0 g.
4. Peptone	20.0 to 25.0 g.

5. Sodium or potassium phosphate	2.0 g.

Preparation:

(1) Chop 500.0 g. of fat and tendon free beef into very small pieces.
(2) Add 1 liter of water and allow to stand over night in the cold.
(3) Press the juice thru a linen cloth by means of a meat press.
(4) Make up the volume to 1000.0 cc. by the addition of water.
(5) Add 5.0 g. NaCl, 2.0 g. sodium or potassium phosphate and 20 to 25 grams of peptone.
(6) Boil for an hour.
(7) Filter thru a linen cloth and then thru paper.
(8) Adjust the reaction slightly alkaline to litmus by the addition of $KHCO_3$ solution, drop by drop.
(9) Boil for 15 minutes.
(10) Allow to settle. Skim off the fat if any be present.
(11) Distribute as desired.

Sterilization: Sterilize at 115 to 120°C. for 30 minutes or by the fractional method.

Use: General culture medium.

Variants: Besson prepared a similar medium as follows:

(1) Remove all fat and tendons from beef and chop into small pieces.
(2) Allow 500.0 g. of (1) to macerate with 1000.0 cc. of cold water for 6, or if one wishes to remove the sugar, 12 hours at 37°C.
(3) Place in an enamelled pot and bring slowly to a boil.
(4) Boil for 10 minutes.
(5) Throw on a thick cloth and press the meat free from juice.
(6) Filter the juice thru moistened paper.
(7) Add 10.0 g. of Chapoteaut's or Defresne's peptone, 5.0 g. NaCl and about 1.0 g. of sodium phosphate.
(8) Boil stirring constantly until solution is complete.
(9) Neutralize or make slightly alkaline to litmus by the addition of soda solution.
(10) Heat at 115 to 117°C. for 5 minutes.
(11) Filter until clear.
(12) Make up to 1000.0 cc. by the addition of distilled water.
(13) Distribute as desired.
(14) Sterilize at 100 to 115°C. for 20 minutes.

Reference: Roux and Rochaix (1911 p. 106), Besson (1920 p. 26).

783. Zielleczky's Phenolphthalein Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Phenolphthalein solution	

Preparation:

(1) Prepare bouillon from 1, 2, 3 and 4. (Exact method not given.)
(2) Make alkaline with Na_2CO_3.
(3) Prepare a mixture of 50.0 cc. of water and 50.0 cc. of absolute alcohol.
(4) Dissolve 1.0 g. of phenolphthalein in (3).
(5) Just before use add 1.0 cc. of (4) to 19.0 cc. of water.
(6) Add 0.7 to 0.8 cc. of (5) to 5.0 cc. of bouillon.

Sterilization: Not specified.

Use: Differentiation of coli and typhoid organisms. Coli decolorized the red medium completely and more thoroly than did typhoid. Coli caused decolorization after 5 to 7 hours.

Reference: Zielleczky (1902 p. 153).

784. Muller's Indicator Infusion Broth

Constituents:

1. Meat Infusion	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.
4. Indicator	

Preparation:

(1) Exact method of preparation of meat infusion from fresh meat not given.
(2) Dissolve 2 and 3 in 1.
(3) Make slightly alkaline.
(4) Add 10 drops (12 drops = 1.0 cc.) of a 1:100 solution of methylene blue, 10 drops of 1:100 solution of rosanalin, 12.0 cc. of a concentrated watery solution of litmus or 10.0 cc. of a 2:100 solution of indigo carmine.

Sterilization: Not specified.

Use: To study the reduction ability of the colon-typhoid group and other bacteria.

Variants: Harvey added one of the following combinations of dyes or indicators to a liter of meat infusion peptone solution prepared as indicated in variant (bb) 634.

(a) Cresol red (1.6% solution in 95.0% alcohol) 1.0 cc.
(b) Brom cresol purple (1.6% solution in 95.0% alcohol) 4.0 cc.
(c) Brom cresol purple, cresol red indicator solution 1.0 cc.
(d) China blue 0.025 g. + rosolic acid or its sodium salt 0.05 g.
(e) China green (0.2% solution) 15.0 cc.
(f) Malachite green 1.0 g.
(g) China blue 0.025 g. + phenol red (phenol sulphone phthalein) 0.01 g.

Reference: Muller (1899 p. 805), Harvey (1921–22 pp. 88–92), Omelianski (1903 p. 4).

785. Robinson and Rettger's Infusion Opsine Solution

Constituents:

1. Beef Infusion	....	1000.0 cc.
2. Opsine	....	20.0 g.

Preparation:

(1) Method of preparation or composition of beef infusion not given.
(2) Dissolve 2 in (1).
(3) Adjust reaction faintly alkaline to litmus.
(4) Distribute in 20.0 cc. lots in 150.0 cc. Erlenmeyer flasks.

Sterilization: Sterilize at 12 to 14 pounds pressure for 15 minutes.

Use: Production of diphtheria toxin by *B. diphtheriae*. The author reported that the addition of 0.1% glucose had little effect. M.L.D. = 0.035 cc.

Variants:

(a) The author added 0.1% glucose with or without 0.5 g. NaCl.
(b) The author added 0.5 g. NaCl.

Reference: Robinson and Rettger (1917 p. 367).

786. Warden, Connell and Holly's Veal Infusion Broth

Constituents:

1. Veal Infusion	....	1000.0 cc.
2. Peptone (Difco proteose)	...	20.0 g.
3. NaCl	....	5.0 g.

Preparation:

(1) Method of preparation of veal infusion not given.
(2) Dissolve 2 and 3 in 1.
(3) Adjust to pH = 7.9 by the addition of NaOH.

Sterilization: Sterilize in the autoclave.

Use: Cultivation of *C. diphtheriae* and *B. megatherium* and to produce large quantities of toxin. Authors reported that a very high grade of toxin was produced.

Variants: The following authors have suggested various methods of preparing this medium:

(a) Dernby and David.
  (1) Immerse 6.0 g. of fresh finely chopped veal in 12 liters of tap water and add 70.0 g. of ordinary distillers yeast.
  (2) Incubate at 36°C. for 16 hours.
  (3) Press thru cheese cloth.
  (4) Add 1.5% Witte's peptone and 0.4% NaCl.
  (5) Adjust to pH = 7.2 or 7.3.
  (6) Boil several minutes.
  (7) Filter while warm thru paper.
  (8) Distribute in liter flasks.
  (9) Sterilize at 110° for 20 minutes.
(b) Dernby and Blanc.
  (1) Chop veal finely and allow to autolyze at 37° for 24 hours. Then boil and filter.
  (2) Dissolve 2.5 g. NaCl and 5.0 g. peptone in (1).
  (3) Adjust to desired pH with NaOH and HCl.
  (4) Sterilize at 110° for 20 minutes.
(c) Harvey.
  (1) Mince finely fat-free veal.
  (2) Add 500.0 g. to 1000.0 cc. tap water.
  (3) Add an 18 hour culture of *B. coli*.
  (4) Incubate 20 hours at 22°C.
  (5) Heat the mixture 2 hours at a temperature not exceeding 50°C.
  (6) Skim off fat on the surface.
  (7) Raise to boiling point.
  (8) Boil 10 minutes.
  (9) Pour the mixture onto a wet, thick, clean cloth.
  (10) Collect the fluid which drains thru the cloth together with that obtained by squeezing the meat in the cloth.
  (11) Filter the fluid collected thru well wetted, thick filter paper.
  (12) Add to the filtrate: peptone 20.0 g., sodium chloride 5.0 g.

(13) Bring the volume up to 1000.0 cc.
(14) Make the reaction 1.2% acid to phenolphthalein.
(15) Steam 30 minutes.
(16) Filter while hot, thru well wetted, thick filter paper.
(17) Distribute the filtrate into test tubes or flasks.
(18) Sterilize.

(d) Brown did not specify the method of preparation of the veal infusion, but specified the use of 10.0 g. of Fairchild's, Witte's, Bacto peptone or aminoids. The initial pH was between 6, 7 and 7. .

References: Warden, Connell and Holly (1921 p. 104), Dernby and David (1921 p. 156), Dernby and Blanc (1921 p. 420), Harvey (1921–22 p. 113), Brown (1921 p. 561).

787. Schoenholz and Meyer's Veal Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Lean veal	500.0 g.
3. Difco peptone	10.0 g.
4. Na_2HPO_4	
5. KH_2PO_4	

Preparation:

(1) Infuse 500.0 g. finely chopped veal in a liter of water on ice over night.
(2) Boil 20 minutes and strain thru cheese cloth and allow to cool.
(3) Filter to remove fat.
(4) Add 3 to (3).
(5) Adjust reaction to pH = 7.4.
(6) Stabilize in Arnold for 30 minutes.
(7) Filter and tube in 10.0 cc. lots.
(8) To each 10.0 cc. tube HCl or NaOH is added to obtain the desired reaction (varying from pH 4.3 to pH 9.0).
(9) Prepare 0.5M solutions of KH_2PO_4 and Na_2HPO_4 and mix in equal volumes.
(10) Add 2.0 cc. of sterile (9) to each tube of sterile (7).

Sterilization: Sterilize (7) in steam on each of 3 consecutive days. Method of sterilization of NaOH, HCl or phosphate solutions not given.

Use: To determine optimum pH values for typhoid bacilli. Optimum growth occurred between pH = 6.8 and 7.0 in a salt-free medium.

Variants: Omit the sodium and potassium phosphate.

Reference: Schoenholz and Meyer (1921 p. 387).

788. Bunker's Veal Infusion Polypeptide Solution

Constituents:

1. Tap water	1000.0 cc.
2. Lean veal	1.0 lb.
3. NaCl	5.0 g.
4. Polypeptides	2.0 g.

Preparation:

(1) Mix chopped veal and water in afternoon and warm to 40°C. in large open kettle.
(2) Inoculate with *B. coli*.
(3) Allow to stand over night.
(4) Heat to coagulate proteins.
(5) Strain and place in flasks.
(6) Heat in the autoclave at 20 pounds for ½ hour.
(7) Add NaCl and polypeptides and NaOH to give pH = 7.8.
(8) Heat in autoclave at 15 pounds for 15 minutes.
(9) Filter thru bibulous filter or thick cloth.
(10) Distribute in amber glass bottles.

Sterilization: Sterilize with steam in usual manner.

Use: Study of production of diphtheria toxin.

Reference: Bunker (1919 pp. 404, 405).

789. Krasnow's et al. Sugar Free Veal Broth

Constituents:

1. Tap water	1000.0 cc.
2. Bacto veal	75.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.

Preparation:

(1) Place 75.0 g. Bacto veal in 1000.0 cc. tap water and heat in the Arnold sterilizer for two hours.
(2) Allow the coagulum thus formed to settle to the bottom of the container. Allow to cool very slowly.
(3) Strain thru a wire sieve.
(4) Dissolve 3 and 4 in (3).
(5) Adjust to pH = 7.9.
(6) Steam in the Arnold for 15 minutes.
(7) Filter.

Sterilization: Sterilize in the Arnold for

30 minutes on each of three successive days.

Use: Cultivation of streptococci.

Reference: Krasnow, Rivkin and Rosenberg (1926 p. 389).

790. Cesaris-Demel's Liver Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Liver (calf)	250.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Litmus	

Preparation:

(1) Chop fresh calf liver into small pieces.
(2) Add to 1000.0 cc. water, 250.0 g. of (1) and infuse for 24 hours.
(3) Press out the infusion and filter.
(4) Boil the liquid for 1 hour at 100°C.
(5) Filter and add 3 and 4.
(6) Filter and neutralize with normal soda solution (usually requires 3.0 cc. solution to obtain the proper reaction).
(7) Place in the autoclave for ½ hour at 115°C.
(8) Filter and add 20.0 cc. of neutral litmus solution.
(9) Tube in 10.0 cc. lots.

Sterilization: Sterilize in the autoclave.

Use: Cultivation of the colon-typhoid group and other organisms.

Variants: This medium has been prepared in a variety of manners. The following authors gave the various listed methods of preparation:

(a) Pfuhl cultivated anaerobes in a medium prepared as follows:
  (1) Pass 500.0 g. of fresh beef liver thru a meat grinding machine and add to 1000.0 cc. of water.
  (2) Allow (1) to stand in the ice box for 1 to 2 hours.
  (3) Boil the mixture for 1 hour.
  (4) Strain thru a straining cloth.
  (5) Add peptone, NaCl and soda solution in the usual manner (amount not given).
  (6) Boil and filter.
  (7) Distribute into test tubes in 10.0 cc. lots and sterilize in the autoclave (time not given).
  (8) To each tube add 1.0 g. of platinum sponge. Glucose (1.0 or 2.0%) may be added if desired.
  (9) Boil the tubes for 10 minutes in the water bath or steamer.
  (10) Cool quickly and inoculate.
  (11) "Hepin" a material from Much and Römer, may replace the platinum sponge. Boil the bouillon and add 1 drop of the sterile Hepin by means of a small pipette.

(b) Kessler prepared the medium as follows and used it as an enrichment medium for the typhoid bacilli from the blood.
  (1) Chop 500.0 g. of beef liver into small pieces and add to 500.0 g. water.
  (2) Extract (1) at about 50°C. in a steamer for 30 minutes.
  (3) Boil for 30 to 45 minutes.
  (4) Filter.
  (5) Make up the filtrate to 500.0 cc.
  (6) Dissolve 5.0 g. peptone (1%) and 2.5 g. NaCl (0.5%) in (5).
  (7) Neutralize with addition of NaOH to litmus.
  (8) Boil 30 minutes in the steamer and readjust the reaction.
  (9) Distribute in 9.0 cc. lots in tubes.
  (10) Sterilize on 3 successive days for 30 minutes each.

(c) Harvey prepared the medium as follows. He stated that other organs may be similarly treated.
  (1) Make a sterilized liver extract in the same way as a meat extract with 1000.0 g. finely minced fresh ox liver and 1000.0 cc. water.
  (2) Prepare a solution of peptone 2.0% and NaCl 1.0%.
  (3) Sterilize the peptone solution.
  (4) Prepare while the solutions are hot, and with sterile precautions: sterilized liver extract 1; sterilized peptone solution 1.
  (5) Distribute with sterile precautions into test tubes.

(d) Goss et al. cultivated *B. chauvoei*, on a medium prepared as follows:
  (1) Grind 500.0 g. beef liver and add 1000.0 cc. water.
  (2) Cook (1) in flowing steam 1 hour.
  (3) Strain thru cheese cloth and cotton.

(4) Add peptone (1.0%) and NaCl (0.5%).
(5) Titrate to pH = 8.2.
(6) Autoclave at 15 pounds pressure for 20 minutes.

(e) Stitt used the medium as a bile substitute. He used 500.0 g. of ground beef liver, 1 liter of water and 1.0% peptone. The procedure was the same as for Stitt's variant of Dunham's Infusion Broth (see variant (gg) 665).

References: Cesaris-Demel (1899 p. 532), Pfuhl (1907 p. 379), Kessler (1911 p. 604), Harvey (1921–22 p. 69), Goss (1921 p. 615), Stitt (1924 p. 47).

791. Owen, Martin and Pitts' Trypsin Beef Tea

Constituents:
1. Bouillon (Beef tea)
2. Trypsin

Preparation:
(1) Add 10.0 cc. of trypsin (Liquor pancreatica, Digestive Ferments Co.) to 90.0 cc. of sterile bouillon (Beef tea).
(2) Distribute in 5.0 to 10.0 cc. lots in sterile test tubes.

Sterilization: Method of sterilization of beef tea not given.

Use: Enrichment medium for blood cultures. Authors report earlier development of staphylococci, pneumococci, streptococci and typhoid bacilli than on "glucose beef tea."

Reference: Owen, Martin and Pitts (1916 p. 198).

792. Kligler's Heart Infusion Broth

Constituents:
1. Peptone phosphate solution.
2. Beef heart.
3. Saline solution.

Preparation:
(1) Exact composition of peptone phosphate solution not given.
(2) Weigh out 2 equal portions of beef heart.
(3) Suspend one portion of (2) in 10 volumes of saline solution and the other in ten volumes of alcohol ether (1:3). The alcohol is added first to desiccate the clots and then the ether.
(4) Filter the saline extract thru Berkefeld candle (length of extraction not given).
(5) Decant the ether extract, evaporate nearly to dryness under suction, and take up the residue in an amount of saline equal to the original volume.
(6) Add 1.0 cc. of (4) or (5) or a mixture of the two to 5.0 cc. of (1).

Sterilization: Method not given.

Use: To show growth accessory substances for pathogenic bacteria. Author reported that the saline extract contained most growth accessory materials while the ether extracts were practically devoid of them.

Reference: Kligler (1919 p. 41).

793. Harvey's Heart Infusion Broth

Constituents: 1. Ox heart Bouillon.

Preparation:
(1) Prepare bouillon 1% acid to phenolphthalein from ox heart which has been "hung" 2 days.
(2) Sow with *B. lactis aerogenes.*
(3) Incubate 48 hours.
(4) Steam 20 minutes.
(5) Make reaction 1% acid to phenolphthalein.
(6) Sow with *B. coli.*
(7) Incubate 48 hours.
(8) Steam 20 minutes.
(9) Test for absence of sugar by cultivation of *B. coli* or *B. lactis aerogenes,* using fermentation tubes.
(10) Leave in a cool place for the growth to sediment.
(11) Filter the supernatant fluid thru thick filter paper or thru a porcelain candle.
(12) Distribute into test tubes.

Sterilization: Method not given.

Use: Sugar free culture medium.

Reference: Harvey (1921–22 p. 113).

794. Park, Willams and Krumwiede's Heart Infusion Broth

Constituents:

1. Water (tap)	1000.0 cc.
2. Heart (Beef)	500.0 g.
3. Peptone	15.0 g.
4. NaCl	5.0 g.
5. Egg	1

Preparation:
(1) Add 500.0 cc. of tap water to 500.0 g.

chopped fat and tendon free beef heart.
(2) Add 15.0 g. of peptone, 5.0 g. NaCl and one well beaten egg to (1).
(3) Heat in a water bath, double boiler or over the open flame, stirring constantly until the color changes to brown (at about 68° to 70°C).
(4) Strain thru a wire sieve or wire gauze. Do not use cheese cloth, cotton or paper.
(5) Heat 500.0 cc. of water to 70°C.
(6) Mix (5) and (4).
(7) Adjust the reaction to +0.2 phenolphthalein or to about pH = 7.4.
(8) Heat in an autoclave at 15 pounds for 30 minutes.
(9) Remove the kettle carefully and set aside for sedimentation to take place or run in Sharples centrifuge.
(10) Decant, if allowed to settle.
(11) Melt.
(12) Tube.

Sterilization: Sterilize in an autoclave at 15 pounds for 30 minutes.

Use: Cultivation of meningococcus.

Reference: Park, Williams and Krumwiede (1924 p. 126).

795. Wroblewski's Suprarenal Capsule Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Suprarenal capsule (ox)	500.0 g.
3. Peptone	15.0 g.
4. NaCl	5.0 g.
5. Soda	

Preparation:
(1) Prepare bouillon in the usual manner using suprarenal capsule (ox) instead of fat free beef.
(2) Dissolve 3, 4 and 5 in (1).

Sterilization: Not specified.

Use: General culture medium.

Reference: Wroblewski (1896 p. 529).

796. Harvey's Organ Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Organs (lungs, liver, spleen, testicles, thymus, placenta or other organs)	500.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.

Preparation:
(1) Mince finely lungs, liver, spleen, kidney, testicles, thymus, placenta or other organs.
(2) Proceed as Harvey's Variant of Schultz's Beef Infusion Peptone Solution (see variant (v) 750).

Reference: Harvey (1921–22 p. 98).

797. Kligler's Blood Clot Infusion Broth

Remove all the serum from blood clots by centrifugation and use blood clots instead of beef heart to prepare medium (see 792.)

798. Hunter's Fish Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Salt-water trout	1000.0 cc.
3. Peptone	15.0 g.
4. Raw fish	

Preparation:
(1) Add 1000.0 cc. water and 15.0 g. peptone to 1000.0 g. finely chopped salt-water trout or weakfish from which skin and bones have been removed.
(2) Heat in Arnold or in water both at 95°–100°C. for an hour.
(3) Strain juice thru cheese cloth, filter thru cotton and adjust reaction to neutral.
(4) Heat in Arnold for 30 minutes at 100° and filter thru paper.
(5) Tube in 10.0 cc. lots and add to each tube about 1.5 raw fish.
(6) If anaerobic cultures are desired, cover the surface with layer of liquid petrolatum.

Sterilization: Sterilize in autoclave for 15 minutes at 15 pounds.

Use: To study the decomposition of fish by organisms resembling colon-aerogenes group. Author reported that some cultures produced foul odor and indol.

Reference: Hunter (1920 p. 543).

799. Harvey's Fish Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Fish flesh	500.0 g.
3. NaCl	26.5 g.
4. KCl	0.75 g.
5. Peptone	5.0 g.

Preparation:

(1) Mince finely the flesh of fish.

(2) Add 500.0 g. to the following solution: sodium chloride 26.5 g.; potassium chloride 0.75 g.; magnesium chloride 3.25 g.; water 500.0 cc.

(3) Heat the mixture 20 minutes over a free flame at a temperature not exceeding 50°C.

(4) Raise the temperature to boiling point.

(5) Boil 10 minutes.

(6) Pour the mixture on to a thick, clean cloth.

(7) Collect the fluid which drains the cloth together with that obtained by squeezing the cloth.

(8) Filter the fluid collected thru well wetted, thick filter paper.

(9) Add peptone, 5.0 g.

(10) Steam 45 minutes.

(11) Bring the volume up to 1000.0 cc. by the addition of water.

(12) Steam 30 minutes.

(13) Filter while hot, thru well-wetted, thick filter paper.

(14) Distribute into flasks or test tubes.

Sterilization: Sterilize in streaming steam.

Use: General culture medium.

Reference: Harvey (1921–22 p. 69).

800. Richardson's Mucosa Infusion Broth

Constituents:

1. Distilled water	500.0 cc.
2. Mucosa, hog intestine	250.0 g.
3. Peptone	5.0 g.
4. NaCl	2.5 g.

Preparation:

(1) Take the small intestine of a hog in fresh condition, without cutting, wash thoroly in running water, until the water runs perfectly clear.

(2) Lay the intestine open and scrape off the mucosa with a glass slide (100.0 g. of mucosa is easily obtained from one hog).

(3) To 250.0 cc. of the mucosa add 500.0 cc. of distilled water.

(4) Boil for 30 minutes and cool.

(5) Boil again, neutralize and boil once more.

(6) Filter.

(7) Boil and 5.0 g. peptone and 2.5 g. NaCl.

(8) Neutralize again and boil for 20 minutes.

(9) Filter.

(10) Tube.

Sterilization: Sterilize using the fractional method.

Use: Attempt to cultivate bodies found in carcinomatous tissue. Author reported no growth.

Reference: Richardson (1900–01 p. 73).

801. Boyer's Bone Infusion Broth

Constituents:

1. Water	900.0 cc.
2. Bones beef ribs	500.0 g.
3. HCl	100.0 cc.
4. Peptone	15.0 g.

Preparation:

(1) Grind beef bones to small pieces.

(2) Mix 500.0 g. of (1) with 900.0 g. water and 100.0 cc. normal HCl.

(3) Allow to stand for 12 to 24 hours.

(4) Heat in the autoclave at 125 to 130°C. for 30 minutes.

(5) Cool and then filter thru linen.

(6) Add 15 parts of peptone to 1000 parts of the liquid.

(7) Neutralize by the addition of a dilute soda solution. (Indicator not specified.)

(8) Heat in the autoclave at 125–130° for 30 minutes.

(9) Filter while hot.

(10) Allow to stand for one day in the cold.

(11) Filter again.

Sterilization: Sterilize at 120° (time not specified).

Use: General culture medium. Author cultivated streptococci, *Streptococcus pyogenes* and other organisms.

Reference: Boyer (1918 p. 230).

802. Ball's Animal Infusion Broth

Use the flesh of guinea pigs as well as other experimental animals instead of beef in the preparation of Dunham's Infusion Broth (779).

Reference: Ball (1919 p. 84).

803. Stutzer's Nitrate Extract Broth

Constituents:

1. Water.
2. Peptone.

3. Meat extract.
4. KNO_3.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Reaction to be slightly alkaline.
(3) Distribute in 10.0 cc. lots.

Sterilization: Method not given.

Use: To study denitrification.

Variants:
(a) von Caron used 6.0 g. Liebig's Meat extract, 6.0 g. peptone and 2.0 g. KNO_3 per liter.
(b) Tanner used 3.0 g. Liebig's meat extract, 10.0 g. Witte's peptone and 3.0 g. KNO_3 per liter.
(c) Committee S. A. B. used 0.1% KNO_3, 3.0 g. beef extract and 5.0 g. peptone per liter.
(d) Percival used 3.0 g. sodium nitrate, 5.0 g. Lemco meat extract and 10.0 g. peptone per liter.

References: Stutzer (1901 p. 83), von Caron (1912 p. 70), Tanner (1919 p. 45), Committee S. A. B. (1920 p. 128), Percival (1920 p. 165).

804. Davis and Ferry's Nitrate Bouillon Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Peptone	20.0 g.
3. Meat extract (Liebig's)	10.0 g.
4. NaCl	4.0 g.
5. K_2HPO_4	3.0 g.
6. $MgSO_4$	0.4 g.
7. KNO_3	0.2 g.

Preparation:
(1) Dissolve 2.0% peptone in 500.0 cc. water.
(2) Dissolve 1.0% Liebig's meat extract in 500.0 cc. water.
(3) Mix (1) and (2).
(4) Dissolve 4, 5, 6 and 7 in (3).
(5) Add sufficient normal NaOH to give a final pH = 8.0 to 8.2.
(6) Steam 15 minutes.
(7) Check the reaction.
(8) Distribute as desired.

Sterilization: Sterilize at 115° for 20 minutes.

Use: Cultivation of *Bact. diphtheriae* and toxin production. Author reported fair growth.

Reference: Davis and Ferry (1919 p. 236).

805. Debrand's Extract Broth

Constituents:

1. Water	1000.0 cc.
2. NaCl	5.0 g.
3. Peptone (Chapoteaut)	10.0 g.
4. Meat extract (Liebig)	5.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Debrand used the medium for the enrichment of the tetanus bacillus. The medium was inoculated with soil that had been heated on each of 3 successive days at 100°C. for 2 minutes. Other investigators used the medium as a general culture medium.

Variants:
(a) Smith (1902) did not specify the type of peptone or meat extract used, and prepared the medium as follows:
  (1) Dissolve 2, 3 and 4 in 1 by heating gently and stirring with a glass rod.
  (2) Boil for 5 to 10 minutes, stirring constantly.
  (3) Filter thru paper.
  (4) Cool and make up to 1 liter.
  (5) Adjust the reaction.
  (6) Boil.
  (7) Filter.
  (8) Distribute
  (9) Sterilize by the fractional method in steam at 100°C. or in the autoclave at 120°C.
(b) Frost (1903) used 3.0 g. Liebig's beef extract, and 1.0% Witte's peptone and prepared the medium as follows:
  (1) Weigh out 3.0 g. of beef extract such as Liebig's.
  (2) Add a liter of distilled water.
  (3) Place in a cooking vessel.
  (4) Add 1.0% peptone (Witte) and 0.5% NaCl to (3).
  (5) Weigh the solution and the vessel.
  (6) Heat to not above 60°C. until the ingredients are in solution.
  (7) Restore the loss in weight by the addition of distilled water.
  (8) Neutralize to phenolphthalein by the addition of normal NaOH.
  (9) Boil for 5 minutes and restore the weight.
  (10) Test for reaction and readjust if necessary.
  (11) Add 0.5% to 1.5% of normal HCl.

(12) Heat until the precipitate appears flaky.
(13) Filter thru moistened filter paper.
(14) Tube.
(15) Sterilize for 20 minutes in a steam sterilizer on 3 consecutive days or in the autoclave at 120° for 20 minutes.

(c) Roux and Rochaix (1911) did not specify the type of peptone used.

(d) Day and Baker (1912–13) specified Lemco meat extract and Witte's peptone. They adjusted the reaction to +1 and cultivated an organism causing ropiness in beer.

(e) Löhnis (1913) used Witte's peptone and adjusted the reaction alkaline to litmus.

(f) Rideal and Walker (1913) used 20.0 g. Liebig's meat extract, 20.0 g. Witte's peptone and 10.0 g. NaCl. The medium was used for the growth of *B. typhosus* in the Rideal-Walker test of disinfectants.
(1) Dissolve the constituents in water by boiling for 30 minutes.
(2) Filter.
(3) Neutralize to phenolphthalein.
(4) Add 15.0 cc. of N/1 HCl. This gives a +1.5 reaction.
(5) Make up to 1 liter.
(6) Filter.
(7) Sterilize (method not given) in 500.0 cc. lots.
(8) Sterilize again on the next day.
(9) Tube in 5.0 cc. quantities into sterile test tubes.
(10) Plug with sterile cotton.
(11) Place in the steam sterilizer for about 30 minutes.

(g) Brussoff (1916) used 5.0 g. of Merck's iron peptone and 2.5 g. NaCl. The medium was used for the cultivation of *Ferribacterium duplex*. A slight yellow membrane was formed after 5 or 10 days incubation.

(h) Berman and Rettger (1916) studied erepsin production by members of the colon-typhoid group using 2.5 g. Liebig's meat extract and did not specify the type of peptone (10.0 g.) used, 5.0 g. NaCl was also used.

(i) Roddy (1917) used 2.0 g. Liebig's beef extract, 5.0 g. NaCl, and 10.0 g. Witte's peptone. The medium was prepared as follows:
(1) Make a paste of the peptone.
(2) Dissolve (1) in 1.
(3) Add 2 and 3 to (2).
(4) Boil and stir for 30 minutes.
(5) Make up the loss due to evaporation by the addition of water.
(6) Bring to the boiling point.
(7) Adjust the reaction as desired.
(8) Filter when hot and again when cool.
(9) Sterilize in the autoclave.

(j) Brussoff (1918) cultivated sludge forms on a medium containing 5.0 g. NaCl, 10.0 g. Witte's peptone, and 10.0 g. Liebig's meat extract in a liter of tap water rich in potassium.

(k) Foster and Randall (1921) studied the changes in pH values of a medium due to autoclaving and standing. They used 5.0 g. NaCl, 10.0 g. Parke-Davis & Co. peptone, 3.0 g. of Liebig's beef extract, or 50.0 g. of Bacto beef in a liter of distilled water.

(l) Harvey used 3.0 to 5.0 g. of Lemco meat extract and prepared the medium as follows:
(1) Add the whites of 2 eggs to 1000.0 cc. water.
(2) Add to the mixture by degrees to make a suspension; Lemco 3 to 5.0 g., peptone 10.0 g., sodium chloride 5.0 g.
(3) Steam or boil 45 minutes.
(4) Filter while hot thru well-wetted, thick filter paper or thru 2 layers of absorbent cotton wool.
(5) Bring the volume up to 1000.0 cc. by the addition of water.
(6) Estimate and adjust to a definite pH value or faintly alkaline to litmus or 1.0% acid to phenolphthalein.
(7) Sterilize in streaming steam or in the autoclave.

(m) Pitfield (1922) used 3.0 g. Liebig's beef extract, 10.0 g. Witte's peptone and 5.0 g. of NaCl per liter. The medium was prepared as follows:
(1) Dissolve the constituents in 1000.0 cc. of water.
(2) Weigh the saucepan and contents and heat to 60°C.

(3) Make up the loss in weight by the addition of water.
(4) Neutralize to litmus.
(5) Boil 5 minutes.
(6) Make up the loss in weight by the addition of water.
(7) Adjust the reaction as desired (+0.5 to +1.5%).
(8) Filter thru paper.
(9) Distribute into flasks or tubes.
(10) Sterilize (method not given).

(n) Dopter and Sacquepee (1921) did not specify the use of Liebig's meat extract, nor did they specify the amount of NaCl or peptone used.

(o) Stitt (1923) prepared the medium as follows:
(1) Place 3.0 g. Liebig's meat extract, 10.0 g. peptone, and 5.0 g. NaCl in a mortar.
(2) Dissolve the white of one or two eggs in 1000.0 cc. of water.
(3) Add (2) little by little to (1) until a brownish solution is obtained.
(4) Pour (3) into the inner compartment of a rice cooker.
(5) Apply heat to the outer compartment containing NaCl or $CaCl_2$.
(6) Bring to a boil and boil for 15 to 20 minutes. Do not stir.
(7) Place the inner compartment on a scales, and its counterpoise and a one kilo weight on the other side. Add water to the medium until the two arms balance.
(8) Filter.
(9) Sterilize in the autoclave at 115°C. for 15 minutes or in the Arnold on 3 successive days.
(10) The reaction rarely exceeds +0.75 (from +0.6 to +0.9) and does not require adjusting.

(p) Manwaring, Boyd and Okami (1923) cultivated *S. cholerae* in a medium containing 10.0 g. peptone, 2.5 g. beef extract, and 5.0 g. NaCl per liter. The reaction was adjusted to be identical to that of Locke's solution (0.015% $NaHCO_3$).

(q) Park, Williams and Krumwiede (1924) suggested the use of 2.0 to 5.0 g. Armour or other commercial beef extracts in addition to Liebig's product. They used 5.0 g. NaCl, but did not specify the type of peptone employed.

(r) Cunningham (1924) prepared the medium as follows:
(1) Weigh out 10.0 g. peptone, 5.0 g. NaCl and 10.0 g. of Liebig's meat extract. Weigh out the extract on a piece of paper.
(2) Place (1) into pot and add 1000.0 cc. of tap water.
(3) Steam for an hour in a double walled pot (one hour from the time the water boils).
(4) Add normal NaOH solution until the liquid turns a piece of moistened tumeric paper dipped into it faintly but distinctly brown. The reaction is acid to phenolphthalein, pH varies from 7.7 to 8.0.
(5) Heat in an autoclave.
(6) Filter thru gray coarse filter paper until clear.
(7) Make up the volume to 1 liter with distilled water.
(8) Tube in 8.0 cc. lots.
(9) Sterilize at 22.5 pounds pressure.

References: Debrand (1900 p. 759), Smith (1902 p. 82), Frost (1903 p. 6), Roux and Rochaix (1911 p. 108), Day and Baker (1912–13 p. 435), Löhnis (1913 p. 14), Rideal and Walker (1913 p. 575), Brussoff (1916 p. 552), Berman and Rettger (1916 p. 537), Roddy (1917 p. 41), Brussoff (1918 p. 205), Foster and Randall (1921 pp. 152, 153), Pitfield (1922 p. 114), Dopter and Sacquépée (1921 p. 118), Stitt (1923 p. 33), Manwaring, Boyd and Okami (1923 p. 307), Park, Williams and Krumwiede (1924 p. 97), Cunningham (1924 p. 11), Besson (1920 p. 30), Harvey (1921–22 p. 68).

806. Berman and Rettger's Extract Proteose Solution

Constituents:

1. Water	1000.0 cc.
2. Proteose	2.5, 5.0, 8.0 g.
3. Beef extract	2.5 or 5.0 g.
4. NaCl	5.0 g.

Preparation:
(1) Prepare proteose by salting out the proteose from commercial peptone (details of the method not given).

(2) Dissolve 2.5, 5.0 or 8.0 g. of (1), 2.5 or 5.0 g. of beef extract and 5.0 g. NaCl in a liter of water.

Sterilization: Not specified.

Use: To study bacterial nutrition.

Reference: Berman and Rettger (1918 pp. 377–380).

807. Guth's Selenium Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Meat extract (Liebig)	10.0 g.
3. Peptone (Witte)	10.0 g.
4. NaCl	5.0 g.
5. Sodium selenicate (Merck)	1.0 to 2.5 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Adjust to neutral using litmus as an indicator.

(3) Dissolve 5.0 g. Merck's sodium selenicate in 50.0 cc. distilled water.

(4) Add normal HCl to (3) until the reaction is neutral to litmus (requires about 20.0 cc.).

(5) Make (4) up to 100.0 cc.

(6) Heat in the autoclave for about 15 minutes.

(7) Tube (1) in 10.0 cc. quantities.

(8) Add 0.3 cc. of (6) to each tube of (7).

Sterilization: Not specified.

Use: Enrichment of typhoid bacilli. Colon bacilli are inhibited.

Reference: Guth (1915–16 p. 490).

808. Berman and Rettger's Salt Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte)	5.0 g.
3. Liebig's beef extract	2.5 g.
4. NaCl	5.0 g.
5. K_2HPO_4	1.0 g.
6. $MgSO_4$	0.2 g.
7. $CaCl_2$	0.1 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Tube in 10.0 cc. lots.

Sterilization: Not specified.

Use: To study bacterial nutrition.

Variants: The authors used Digestive Ferments, Park Davis, Eimer and Amend's peptone or Arlington Chemical Company's amenoids instead of Witte's peptone.

Reference: Berman and Rettger (1918 pp. 371–377).

809. Berman and Rettger's Trypsinized Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Beef extract	2.5 g.
3. NaCl	5.0 g.
4. Peptone (Witte)	

Preparation:

(1) Prepare peptone digest by digesting at 45°C. for several hours solutions of Witte's peptone with commercial trypsin. Then sterilize and add extract and NaCl. The amount of peptone was not specified.

(2) Tube in 10.0 cc. lots.

Sterilization: Method not given.

Use: To study bacterial nutrition. Authors reported that more nitrogen was available in this medium than in similar undigested media.

Reference: Berman and Rettger (1918 pp. 383–384).

810. Olszewski and Köhler's Trypsinized Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. Liebig's meat extract	5.0 g.
4. NaCl	5.0 g.
5. Physiological salt solution	3000.0 g.

Preparation:

(1) Prepare ordinary nutrient bouillon using 10.0 g. peptone, 5.0 g. Liebig's meat extract and 5.0 g. NaCl.

(2) Make alkaline by the addition of 7.0 cc. N/L soda solution after neutralizing to litmus.

(3) Boil.

(4) Cool to 40°C. and add 0.2 g. trypsin, 10.0 cc. chloroform and 5.0 cc. toluol.

(5) Place in a glass stoppered flask and place in the incubator for 24 to 48 hours, shaking occasionally.

(6) Filter thru a moistened folded filter paper.

(7) For use dilute one part of this stock solution with 3 parts physiological salt solution.

Sterilization: Not specified.

Use: Water analysis and indol production. Author tested for indol by adding 10.0 drops of Ehrlich's reagent and 10.0 drops of a saturated watery solution of potassium persulphate to a tube after incubation.

Variants: Frieber specified the use of Grübler's trypsin and did not give the exact amounts of chloroform or toluol used.

References: Olszewski and Köhler (1923 p. 5), Frieber (1921–22 p. 275).

811. Bacto Cooledge Broth (Dehydrated)

Constituents:

1. Water	
2. Beef extract (Bacto)	3.0 g.
3. Peptone (Bacto)	10.0 g.
4. NaCl	5.0 g.
5. Dibromthymolsulphonephthalein	0.032 g.

Preparation:

(1) Dissolve 18.0 g. of Bacto Cooledge Broth, (dehydrated) in 1000.0 cc. of distilled water. The formula for the broth is given above.

(2) Reaction pH = 7.0±.

Sterilization: Sterilize in the usual manner.

Use: General culture medium.

Reference: Digestive Ferments Co. (1925 p. 13).

812. Heinemann's Bouillon

Constituents:

1. Water (tap)	300.0 cc.
2. Peptone	3.0 g.
3. Beef extract	1.0 g.

Preparation:

(1) Weigh a saucepan and measure 300.0 cc. tap water into it.

(2) Dissolve 3.0 g. of peptone in (1) when hot.

(3) Make up the loss due to evaporation by adding water.

(4) Dissolve 1.0 g. of beef extract in (3).

(5) Adjust the reaction to alkaline to litmus or neutral to phenolphthalein and add 0.5% normal HCl.

(6) Distribute into Erlenmeyer flasks.

(7) Autoclave at 120° for 10 minutes.

(8) Store for 24 hours in a cool place.

(9) Filter.

(10) Tube.

Sterilization: Autoclave at 120° for 5 minutes.

Use: General culture medium.

Variants:

(a) Committee S. A. B. dissolved 3.0 g. beef extract and 5.0 g. peptone in a liter of distilled water. The medium may be clarified using egg white. Adjust from pH = 6.6 to 7.4.

(b) Ball, Tanner, Levine (A. P. H. A.) and Park, Williams and Krumwiede used 3.0 g. beef extract and 5.0 g. peptone and adjusted the reaction to +1, if the reaction does not fall between +0.5 and +1.0.

(c) Brown used 3.0 or 5.0 g. of Liebig's meat extract and 10.0 or 20.0 g. of Fairchild's peptone or 10.0 g. of Witte's peptone or 10.0 g. of Bacto peptone. Initial pH from 7.1 to 7.4.

References: Heinemann (1905 p. 25), Committee S. A. B. (1918 p. 115), Ball (1919 p. 76), Tanner (1919 p. 44), Levine (1921 p. 109), Brown (1921 p. 561), Park, Williams and Krumwiede (1924 p. 131).

813. Wyant's Bouillon Cube Broth

Constituents:

1. Water	1000.0 cc.
2. Bouillon cubes	3.0 to 5.0 g.
3. Peptone	10.0 g.

Preparation:

(1) Dissolve 2, and 3 in boiling tap water.

(2) Cool liquid to coagulate fats.

(3) Filter cold.

(4) Tube.

Sterilization: Sterilize at 15 pounds in autoclave.

Use: Substitute for beef extract or meat in nutrient media. A very satisfactory substitute for meat. Can also be used in special media omitting the peptone.

Reference: Wyant (1920 p. 189).

814. Heinemann's Nitrate Broth

Constituents:

1. Bouillon	1000.0 cc.
2. KNO_3	5.0 g.

Preparation: (1) Dissolve 2 in 1.

Sterilization: Not specified.

Use: To study denitrification.

Variants:

(a) Sewerin used 3.0, 6.0, 7.0, 8.0 or

9.0 $NaNO_3$ instead of 5.0 g. KNO_3 to bouillon.

(b) Löhnis added about 0.1% $NaNO_3$ to bouillon.

(c) Percival added 3.0 g. $NaNO_3$ to bouillon.

References: Heinemann (1905 p. 131), Sewerin (1909 p. 349), Löhnis (1913 p. 98), Percival (1920 p. 165).

815. Makgill's Indicator Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Neutral red (saturated aqueous solution)	10.0 cc.

Preparation: (1) Add 1.0% of a saturated solution of neutral red to nutrient bouillon.

Sterilization: Not specified.

Use: Detection of *Bacillus coli* in water analysis. *Bacillus coli* gave a canary yellow color to the medium. Different authors have added various indicators for other special purposes. These will be indicated under variants.

Variants:

(a) Gordon reported that *Streptococcus brevis* decolorized bouillon containing 2.0 cc. of a 2.0% watery solution of neutral red per liter while *Streptococcus longus* did not decolorize the medium.

(b) Heinemann added 10.0% of a 1.0% litmus solution to nutrient bouillon.

(c) Calandra added 4 drops of a 1.0% aqueous solution of Brilliant cresyl blue to 10.0 cc. bouillon. He reported that typhoid bacilli caused no change. *B. coli* after 24 hours gave a blue colored layer about ½ cm. high on the upper surface. After 48 hours the blue color persisted in the upper layer. The remainder of the tube was decolorized.

(d) Calandra added 3 drops of a 1.0% congo red solution to 10.0 cc. of bouillon. He reported that typhoid caused no change in color after 24 hours. After 24 hours, *B. coli* gave a strawberry red color. Both typhoid and *B. coli* were red after 48 hours.

(e) Calandra added 3 drops of a 1.0% Kuhne alkali blue to 10.0 cc. of bouillon. He reported that after 48 hours typhoid had not changed color. Coli bacilli formed a greenish blue zone of about 1 cm. on the surface of the bouillon.

(f) Signorelli added 0.5 cc. of a 1.0% dahlia solution, 10.0 cc. of a 1.0% erythrosin solution or 10.0 cc. of a 1.0% safranin solution to 100.0 cc. of bouillon. He studied the adsorption of dye by the cholera vibrio. He reported that the organisms developed slowly. After 24 to 36 hours the colored mass of bacteria settled to the bottom and the medium was decolorized after a week. If the dye was added after the culture had developed the vibrio absorbed the dye. With erythrosin and safranin the bacteria were well colored, but the medium was not decolorized.

(g) Groenewege added 10.0 drops of methylene blue to 150.0 cc. of bouillon. The medium was used to study the reduction of *Phytobacter lycopersicum* n. sp. the cause of tomato rot. Partial reduction occurred.

(h) Botez added 2.0 cc. of a 5.0% methyl violet solution to 100.0 cc. of bouillon. He reported that *B. typhosus* grew well. Did not decolorize (reduce) methyl violet. Para typhoid A changed the color to a pale violet. Paratyphoid B completely decolorized the medium after 48 hours. *B. coli* completely decolorized the medium after 48 hours. *B. fecal alcaligenes* did not reduce methyl violet. *B. Shiga* did not grow. Cholera vibrio grew slowly and did not change the color after 10 days. *B. pyocyaneus* reduced methyl violet.

(i) Tanner added 2.0 cc. of 1.0% neutral red solution to 1000.0 cc. of bouillon containing 1.0% peptone and 0.5% NaCl.

(j) Heinemann added 2.0 to 3.0 cc. of a 2.0% solution of Höchst's 120″ malachite green to bouillon, alkaline to litmus.

References: Makgill (1901 p. 431), Gordon (1904 p. 271), Heinemann (1905 p. 128), Calandra (1910 pp. 570, 571), Signorelli (1912 p. 472), Groenewege (1913 p. 26), Botez (1915 p. 489), Tanner (1919 p. 46), Heinemann (1922 p. 35).

816. Thoinot's Arsenious Acid Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Arsenious acid	0.1 g.

Preparation:

(1) Prepare peptone bouillon.

(2) Add 0.01% arsenious acid to (1).

Sterilization: Not specified.

Use: Differentiation between colon bacillus and the Eberth bacillus. Author reported that the Eberth bacillus did not grow in a medium containing 0.01% arsenious acid. Coli bacillus grew well even in a medium containing 1.5% arsenious acid.

Reference: Thoinot (1898 p. 126).

817. Omeliansky's Dilute Bouillon

Constituents:

1. Distilled water	50.0 cc.
2. Bouillon	50.0 cc.
3. Gypsum	5.0 g.

Preparation:

(1) Method of preparation of bouillon not given.

(2) Dilute 50.0 cc. alkaline bouillon with an equal volume of distilled water.

(3) Add 5.0 g. gypsum to (2).

Sterilization: Not specified.

Use: To study nitrification. The author reported that using a mixture of *Bac. ramosus*, *Nitrosomonas* and *Nitrobacter*, ammonia, nitrites and nitrates were formed. Using *Bac. ramosus* and *Nitrosomonas*, ammonia and nitrites were formed. Using *Bac. ramosus* and *Nitrobacter* only ammonia was formed. Using *Nitrosomonas* and *Nitrobacter* there was only organic nitrogen present.

Variant: Omeliansky omitted the gypsum and added 10.0 cc. alkaline bouillon to 40.0 cc. distilled water.

Reference: Omeliansky (1899 p. 487).

818. Muller and Malvoz's Iodine Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. $CaCO_3$	50.0 g.
3. Sodium hyposulphite.	
4. KI.	
5. Iodine.	

Preparation:

(1) Add 5 parts pure $CaCO_3$ to each 100 parts of ordinary bouillon. Method of preparation of bouillon not given. Mix well.

(2) Distribute in 90.0 cc. lots in 150 or 200.0 cc. flasks.

(3) Make 50.0 g. of crystalline sodium hyposulphite up to 100.0 cc. by the addition of water.

(4) Mix thoroly in a mortar 20.0 g. KI and 25.0 g. iodine, and add enough water to make 100.0 cc.

(5) To each 90.0 cc. of sterile (2) add 10.0 cc. of sterile (3) and 2.0 cc. of sterile (4). Mix well.

(6) Distribute in tubes or flasks.

Sterilization: Sterilize (2) and (3) at 100° for 30 minutes. Sterilize (4) by itself (method not given).

Use: Enrichment medium for typhoid group. *B. coli* and other forms are inhibited.

Reference: Muller and Malvoz (1923 p. 435).

819. Fremlin's Ammonium Sulphate Bouillon

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2SO_4$	1.0 g.
3. Potassium phosphate	1.0 g.
4. $MgCO_3$	10.0 g.
5. Peptone beef broth.	0.1 to 100.0 cc.

Preparation:

(1) Dissolve 2 and 3 in part of 1.

(2) Dissolve 4 in the remainder of 1.

(3) Mix sterile (1) and sterile (2) and add various amounts of sterile 5.

Sterilization: Method not specified.

Use: To study nitrogen oxidation by nitroso bacteria.

Reference: Fremlin (1903 p. 368).

820. Stitt's Carbonate Bouillon

Constituents:

1. Bouillon.
2. $CaCO_3$.

Preparation: (1) Add small fragments of marble ($CaCO_3$) to bouillon.

Sterilization: Not specified.

Use: General culture medium.

Reference: Stitt (1921 p. 35).

821. Dalimier and Lancereau's Opsine Bouillon

Constituents:
1. Bouillon.
2. Opsine.

Preparation:
(1) Method of preparation of bouillon not specified. Reaction alkaline.
(2) Add opsine. (Opsine is a commercial mixture of amino acids.)

Sterilization: Method not given.

Use: General culture medium. Authors reported the growth of staphylococci, typhoid and paratyphoid bacilli, *Micrococcus melitensis*, *B. prodigiosus*, *Vibrio cholerae*, pneumococci and others.

Variants: The authors suggested the addidion of glycerol.

Reference: Dalimier and Lancereau (1913 p. 419).

822. Frieber's Trypsinized Bouillon (Committee S. A. B.)

Constituents:

1. Bouillon	1000.0 cc.
2. Physiological salt Solution	3000.0 cc.
3. Trypsin	0.2 g.

Preparation:
(1) Add 0.2 g. trypsin to a liter of peptone bouillon.
(2) Add chloroform and toluol.
(3) Incubate for 24 to 48 hours at 37°C.
(4) Filter.
(5) Dilute with 3 parts physiological salt solution.

Sterilization: Method not given.

Use: To determine the production of indol.

Reference: Committee Society American Bacteriologists (1923 p. 31).

823. Wilcox's Glucose Veal Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Veal (Market)	1.0 lb.
3. Peptone (Witte)	10.0 g.
4. Glucose (anhydrous)	10.0 g.
5. NaCl	5.0 g.

Preparation:
(1) Add water to chopped veal and let stand in ice chest over night.
(2) Place infusion over free flame and heat to 45°C. for 1 hour.
(3) Boil briskly for ½ hour.
(4) Strain broth thru cheese cloth.
(5) Measure broth and add 3, 4 and 5.
(6) Boil until 3, 4 and 5 are in solution.
(7) Correct to +1, phenolphthalein as indicator, with N/10 NaOH.
(8) Filter thru 2 layers absorbent cotton directly into 2 liter Erlenmeyer flask, leaving only enough space to allow for expansion of broth in Arnold.

Sterilization: Sterilize in Arnold 90 minutes on first day and 1 hour on second day.

Use: Study toxin production by B. tetani and preliminary cultures for inoculation in toxin broth.

Variants:
(a) Walbum studied diphtheria toxin production on a veal infusion medium containing 1.5% Witte peptone, 0.5% NaCl and 0.2% glucose. The medium was sterilized unadjusted, and the sterile medium adjusted to pH = 7.0 by adding sterile calcinated soda. He reported that the addition of small amounts of manganese chloride (final concentration 0.01 molar) in many cases markedly increased the toxin production.
(b) Park, Williams and Krumwiede prepared the medium as above, but did not give the adjustment of reaction and heated for 90 minutes at 90°C. instead of 90 minutes in Arnold.
(c) Park, Williams and Krumwiede used a similar medium for the production of diphtheria toxin. This medium was prepared as follows:
  (1) Mix 20 pounds of lean chopped veal with 20 liters of tap water.
  (2) Place in the ice box over night.
  (3) Strain the next morning.
  (4) When chilled add 10.0 cc. of a 24 hour culture of *B. coli* for each liter and incubate at 37°C. over night.
  (5) In the morning add 1 egg for each 2 liters.
  (6) Boil 20 minutes and strain.
  (7) Adjust the reaction to 0.5 acid to phenolphthalein.
  (8) Add 1.0% Park Davis & Co. peptone, and 0.5% NaCl and heat to boiling to dissolve.
  (9) Readjust the reaction.
  (10) Filter thru paper and cotton.

(11) Distribute 800.0 cc. in 2 liter flasks.
(12) Autoclave for 30 minutes at 15 pounds pressure.
(13) Add 8.0 cc. of a 10.0% glucose solution, sterilized in the Arnold on each of 3 successive days, for 30 minutes to each flask.

References: Wilcox (1916 p. 334), Walbum (1922 p. 25) taken from (1922 p. 109), Park, Williams and Krumwiede (1924 pp. 132, 133).

824. Park, Williams and Krumwiede's Glycerol Veal Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Veal	1.0 lb.
3. Peptone (Fairchild's (1.0%).	10.0 g.
4. NaCl (0.5%)	5.0 g.
5. Glycerin (5.0%)	50.0 g.

Preparation:
(1) Mince 1.0 pound of lean veal free from fascia with 1 liter of water.
(2) Macerate over night at room temperature.
(3) Heat to 45 to 48° for one hour.
(4) Boil up strongly.
(5) Strain thru cheese cloth.
(6) Add 1.0% Fairchild's peptone and 0.5% NaCl.
(7) Boil to dissolve the peptone.
(8) Titrate and adjust the reaction to +2.5 to phenolphthalein (room temperature) (about pH = 6.0 to 6.4).
(9) Autoclave for 15 minutes at 15 pounds pressure, to clear.
(10) Filter thru cotton and paper.
(11) Measure the filtrate and add 5.0% glycerol.
(12) Distribute in 250.0 cc. quantities in each quart Blake bottle or liter flask.

Sterilization: Sterilize in the autoclave for 30 minutes at 15 pounds pressure.

Use: Cultivation of *B. mallei* and malein production for diagnosis of glanders. Cultivation of tubercle bacilli and tuberculin production.

Variants: The authors used the medium with a different reaction to cultivate tubercle bacilli and for the production of tuberculin. This medium was adjusted to +1.3 to phenolphthalein (pH about 7.1) and the final reaction +1.5 to phenolphthalein or pH about 6.9.

Reference: Park, Williams and Krumwiede (1924 p. 133).

825. Reeser's Potato Veal Infusion Broth

Constituents:

1. Distilled water	3000.0 cc.
2. Potato	
3. Peptone	30.0 g.
4. NaCl	15.0 g.
5. Glycerin	150.0 g.
6. Veal	1500.0 g.

Preparation:
(1) Peel potatoes and carefully remove all eyes.
(2) Cut into thin slices.
(3) Allow to stand in water for a day, renewing the water occasionally.
(4) Soak in distilled water over night.
(5) Pour off the water and heat the pieces of potato for $\frac{3}{4}$ hour in autoclave at 1.5 atmospheres pressure.
(6) Add 3.0 liters of distilled water.
(7) After sterilization allow the potatoes to stand in water for one day.
(8) Filter the well extracted potato slices and make the filtrate up to 3 liters.
(9) Grind 1500.0 g. of lean veal in a meat chopping machine.
(10) Add (8) to (9) and place in the ice box over night.
(11) Filter thru a linen towel.
(12) Add water to make 3 liters.
(13) Boil 10 minutes.
(14) Filter thru a flannel cloth.
(15) Add 1% peptone and 0.5% NaCl which one has prepared a paste of with a little of the hot liquid.
(16) Add 5.0% glycerin.
(17) Neutralize with N/1 Na_2CO_3 using litmus as an indicator.
(18) Add 3.0 cc. N/1 Na_2CO_3 per liter to give a slight alkaline reaction or adjust the reaction so that 5.0 cc. of the bouillon to which phenolphthalein has been added, will be colored weakly red when 0.4 cc. N/1 NaOH or Na_2CO_3 be added.
(19) Sterilize for 2 hours, method not given.
(20) If not transparent after sterilization filter thru paper or flannel.
(21) Distribute in sterile Roux flasks.

Sterilization: Sterilize (6) by autoclaving for 45 minutes at 1.5 atmospheres of pressure. Sterilize (19) for two hours, method not given.

Use: Cultivation of tubercle bacilli and preparation of tuberculin.

Reference: Reeser (1908 p. 152).

826. Mellon's Serum Veal Infusion Broth

Constituents:

1. Veal Infusion Broth	1000.0 cc.
2. Glucose (1.0%)	10.0 g.
3. Serum (human or rabbit).	

Preparation:

(1) Prepare a 1.0% glucose veal infusion broth.
(2) Adjust (1) to +1.6 to phenolphthalein.
(3) Add a few drops of sterile human or rabbit serum to each tube of (2).

Sterilization: Not specified.

Use: Cultivation of diphtheroids.

Reference: Mellon (1916 p. 84).

827. Albert and Kelman's Blood Veal Infusion Broth

Constituents:

1. Veal Infusion Broth	1000.0 cc.
2. Blood, rabbit defibrinated	100.0 cc.

Preparation:

(1) Prepare 1 liter of veal infusion broth.
(2) Adjust to neutral to phenolphthalein.
(3) Distribute in 50.0 cc. lots in Erlenmeyer flasks.
(4) Add 5.0 cc. of sterile defibrinated rabbit blood to each flask.
(5) Heat the mixture over a water bath at 75°C. until the blood coagulates and settles on standing (3 to 5 minutes after reaching the temperature).

Sterilization: Method not given.

Use: Cultivation of *B. influenzae* and toxin production. After incubation at 37°C. for 24 hours, centrifuge to remove the coarse particles and then filter thru a mandler candle filter.

Reference: Albert and Kelman (1919 p. 434).

828. Thjötta and Gundersen's Blood Veal Infusion Solution

Constituents:

1. Veal broth.
2. Sodium phosphate (secondary) (Na_2HPO_4).
3. Blood.

Preparation:

(1) Prepare veal broth but use 0.2% Na_2HPO_4 instead of NaCl.
(2) Adjust to pH = 7.8.
(3) Add 10.0 cc. of blood to 100.0 cc. of sterile (2).
(4) Incubate at 37°C.

Sterilization: Sterilize (2) at 115° for 30 minutes.

Use: Isolation of a streptothrix from the blood.

Reference: Thjötta and Gundersen (1925 p. 1).

829. Robinson and Meader's Liver Veal Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Lean veal	500.0 g.
3. Peptone	20.0 g.
4. NaCl	5.0 g.
5. Liver (Fresh sterile guinea pig).	

Preparation:

(1) Mince lean veal fine, and soak in water in ice box for 18 to 24 hours.
(2) Filter thru wire gauze and heat to 80° for 5 minutes.
(3) Cool and remove fat from the surface.
(4) Add 3 and 4 to (3).
(5) Adjust to 0.3% acid to phenolphthalein.
(6) Boil 5 minutes and adjust again if necessary.
(7) Filter thru wire gauze and sediment allowed to settle.
(8) Distribute in 125.0 cc. lots in 300.0 cc. Florence flasks.
(9) Kill a large guinea pig by a blow on the head and plunge the guinea pig into a 0.5% lysol solution for a few minutes.
(10) Open the body cavity aseptically and remove the liver.
(11) Introduce a piece of liver slightly larger than a 25 cent piece into each flask containing 125.0 cc. sterile medium.

Sterilization: Sterilize (8) by heating at 15 pounds pressure for 15 minutes.

Use: Cultivation of diphtheria bacilli, and production of toxin by the Park Williams strain #8. Contamination from liver may be detected in smear preparations at the time of testing the toxin and also by the odor of the broth.

Reference: Robinson and Meader (1920 p. 107).

830. Starin and Dack's Casein Digest Veal Infusion Broth

Constituents:

1.	Veal infusion	1000.0 cc.
2.	Peptone	10.0 g.
3.	NaCl	5.0 g.
4.	Casein digest (Wolf's)	50.0 g.

Preparation:

(1) Method of preparation or composition of veal infusion not given.

(2) Dissolve 2, 3 and 4 in (1). The preparation of Wolf's casein digest was not given by the authors. It was prepared according to Kahn's method. See 648.

(3) Filter to remove any precipitated material.

(4) Adjust reaction to pH = 7.8 to 8.0

Sterilization: Method not given.

Use: Cultivation of *Clostridium botulinum.*

Reference: Starin and Dack (1923 p. 173).

831. Bacto Veal Infusion Medium (Dehydrated)

Constituents:

1.	Distilled water.	
2.	Veal infusion	8.0 g.
3.	Peptone, Bacto	10.0 g.
4.	Agar, Bacto	1.0 g.

Preparation:

(1) Dissolve 19.0 g. of Bacto Veal Infusion Medium (Dehydrated) in 1000.0 cc. of distilled water by boiling. (Low agar content according to Hitchens.

(2) Restore the loss in weight if necessary.

(3) If sterilized at 15 pounds for 20 minutes pH = 7.5±.

Sterilization: Sterilize at 15 pounds for 20 minutes.

Use: General culture medium.

Reference: Digestive Ferments Co. (1925 p. 11).

832. Bruschettinis' Blood Egg Broth (Klimmer)

Constituents:

1.	Veal Infusion Broth	1000.0 cc.
2.	Blood, defibrinated, rabbit or dog	100.0 cc.
3.	Egg yolk.	

Preparation: (1) Add 50.0 cc. of egg yolk and 100.0 cc. of dog or rabbit defibrinated blood to 1000.0 cc. of veal infusion broth.

Sterilization: Method not given.

Use: Cultivation of tubercle bacilli.

Reference: Klimmer (1923 p. 224).

833. Jackson and Muer's Glucose Liver Infusion

Constituents:

1.	Water	1000.0 cc.
2.	Liver, beef	500.0 g.
3.	Peptone (Witte)	10.0 g.
4.	Glucose	10.0 g.
5.	K_2HPO_4	1.0 g.

Preparation:

(1) Chop 2 into small pieces and add 1. Weigh the infusion and container.

(2) Boil slowly for 2 hours in a double boiler, starting cold and stirring it occasionally.

(3) Make up the loss in weight by evaporation and strain thru a wire strainer.

(4) To the filtrate add 3, 4 and 5. Weigh the infusion and container.

(5) After warming this mixture in a double boiler and stirring it for a few minutes to dissolve ingredients, titrate with N/20 sodium hydrate, using phenolphthalein as an indicator and neutralize with normal sodium hydrate.

(6) Boil vigorously for 30 minutes in a double boiler and 5 minutes over a free flame with constant stirring to prevent the caramelization of the dextrose.

(7) Make up any loss in weight by evaporation and filter thru cotton flannel and filter paper.

Sterilization: Tube and sterilize in an autoclave for 15 minutes.

Use: Presumptive test for gas formers in water. Author reported that this medium gave a larger amount of attenuated forms, had greater rejuvinating power, gave fewer abnormalities and greater and more rapid gas production than did the usual dextrose broth. *B. coli* produced gas.

Variants:
- (a) Harvey prepared a similar medium as follows:
  - (1) Mince finely ox liver.
  - (2) Add 500.0 g. to 1000.0 cc. distilled water.
  - (3) Heat the mixture 20 minutes at a temperature not exceeding 50°C.
  - (4) Skim off fat floating on surface.
  - (5) Raise the temperature to boiling point.
  - (6) Boil 10 minutes.
  - (7) Pour the mixture on to a wet, thick, clean cloth.
  - (8) Collect the fluid which drains thru the cloth together with that obtained by squeezing the meat in the cloth.
  - (9) Filter the fluid collected thru well-wetted, thick filter paper.
  - (10) Add to the filtrate, peptone 10.0 g.
  - (11) Bring the volume up to 1000.0 cc. by the addition of water.
  - (12) Adjust the reaction.
  - (13) Steam 30 minutes.
  - (14) Filter, while hot, thru well-wetted, thick filter paper.
  - (15) Dissolve 10.0 g. glucose and 1.0 g. K_2HPO_4 in the filtrate.
  - (16) Harvey further modified the medium in that he added 1.0 cc. of defibrinated rabbit blood to each 10.0 cc. of the medium.
  - (17) Sterilization not specified.
- (b) Klimmer prepared a medium as follows:
  - (1) Boil finely chopped liver with a double amount of water.
  - (2) Add 1.0% peptone and 0.5% NaCl to the juice of (1).
  - (3) Neutralize to phenolphthalein by the addition of NaOH.
  - (4) Boil.
  - (5) Filter.
  - (6) Add 2.0% glucose.
  - (7) Cut the boiled liver in pieces 1–2 ccm.
  - (8) Sterilize (7) (Method not given).
  - (9) When ready for use, add about 4 pieces of (8) to a series of tubes and add (6) until the pieces of liver are covered to a depth of 3 cm.
- (10) Sterilize (method not given).
  - (11) Add a 2 cm. layer of sterile paraffin to each tube after inoculation.

References: Jackson and Muer (1911 p. 290), (1911 p. 727), Levine (1921 p. 110), Harvey (1921–22 p. 110), Klimmer (1923 p. 201).

834. Haslam's Brain Liver Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Beef liver	500.0 g.
3. Brain	500.0 g.
4. Peptone (1.0%)	10.0 g.
5. NaCl (0.5%)	5.0 g.

Preparation:
- (1) Grind liver and to each liter of water add 500.0 g. liver.
- (2) Bring to boil slowly and cook for about 30 minutes or until the liquid is clear.
- (3) Pour off the clear broth, and add 1.0% peptone and 0.5% NaCl.
- (4) Adjust to pH = 8.0.
- (5) Grind brain, using the coarse grinder and cook at 3 pounds pressure for about one and one half hours.
- (6) Fill flasks ¾ full with mixture of brain tissue and liver broth (amounts of each not given).

Sterilization: Autoclave at 6 pounds pressure for three hours.

Use: Cultivation of *B. chauvoei* and streptococci.

Variants:
- (a) Gross, Barabin and Haines prepared the medium as follows:
  - (1) Grind beef liver and brain separately.
  - (2) To ground liver add 1000.0 cc. water.
  - (3) Cook ground brain and (2) in flowing steam for 1 hour.
  - (4) Strain liver broth thru cheese cloth and cotton.
  - (5) Add peptone (1.0%) and NaCl (0.5%).
  - (6) Titrate to pH = 8.2.
  - (7) Tube, two parts (6) to one part brain.
  - (8) Autoclave for one hour at 15 pounds pressure.
- (b) Long and Cornwell attempted to cultivate streptococci from a measles patient on a medium prepared as follows:
  - (1) Heat 500.0 g. of ground fat free beef

liver in 1000.0 cc. of water in flowing steam for two hours.
(2) Add 10.0 g. peptone and 5.0 g. NaCl.
(3) Adjust to pH = 8.2.
(4) Strain through gauze.
(5) Free beef brain from blood and membrane and make a mixture of medium consistency of 500.0 g. brain and 200 to 300.0 cc. water.
(6) Heat (5) in flowing steam for two hours.
(7) Mix 120.0 cc. of (4) with 60.0 cc. of (6).
(8) Final reaction, following sterilizing pH = 7.6.
(9) Autoclave at 15 pounds pressure for one hour.

References: Haslam (1920 p. 540), Goss, Barabin and Haines (1921 p. 615), Long and Cornwell (1927 p. 409).

835. Mueller's Heart Infusion Aminoid Solution

Constituents:

1. Water	600.0 cc.
2. Heart, beef	1.0 lb.
3. Aminoids	10.0 g.
4. NaCl	10.0 g.
5. $MgSO_4$	0.4 g.
6. $CaCl_2$	0.2 g.
7. K_2HPO_4	2.0 g.
8. Glucose	2.0 g.
9. Phenol red (0.02% soln.)	80.0 cc.

Preparation:

(1) Prepare a beef heart infusion by heating 1 pound of chopped beef heart with 500.0 cc. of water to the boiling point. Strain and filter.
(2) Decolorize by boiling for 25 minutes with 10.0% "Norit" a commercial grade of wood charcoal.
(3) Filter thru paper.
(4) Dissolve 10.0 g. aminoids in 100.0 cc. of water.
(5) Precipitate aminoids from (4) with 100.0 cc. of 10.0% solution of $HgSO_4$ in 5.0% H_2SO_4.
(6) Allow to stand over night and filter on a Buchner funnel and wash with water.
(7) Suspend filtrate in water, make slightly alkaline with $Ba(OH)_2$ and decomposed with H_2S with warming.
(8) Filter off the precipitated HgS and free the heavily pigmented filtrate from Ba with H_2SO_4. Dilute to 100.0 cc.
(9) Dissolve 4, 5, 6, 7, 8 and 9 in 1000.0 cc. of water.
(10) Make the following mixture:

Decolorized infusion (2)	25.0 cc.
Glucose salt solution (9)	25.0 cc.
$HgSO_4$ precipitate fraction (8)	0.25 or 2.5 cc.

(11) To the remainder of the precipitate fraction add 10.0 cc. of a 10.0% solution of $HgSO_4$ and allow to stand over night.
(12) Filter on Buchner funnel.
(13) Free precipitate and filtrate from H_2SO_4 and Hg by making slightly alkaline with $Ba(OH)_2$ and bubbling in H_2S.
(14) Free from Ba with H_2SO_4 and dilute to 100.0 cc.
(15) Make the following mixtures:

(a)	Decolorized infusion (2)	25.0 cc.
	Glucose salt solution (9)	25.0 cc.
	$HgSO_4$ filtrate fraction (14)	0.5 or 1.0 cc.
(b)	Decolorized infusion (2)	25.0 cc.
	Glucose salt solution (9)	25.0 cc.
	$HgSO_4$ filtrate fraction (14)	1.0 cc.
	$HgSO_4$ precipitate fraction (8)	1.0 cc.
(c)	Decolorized infusion (2)	25.0 cc.
	Glucose salt solution (9)	25.0 cc.
	$HgSO_4$ filtrate fraction (14)	0.5 or 1.0 cc.
	$HgSO_4$ precipitate fraction (8)	0.5 or 1.0 cc.

(16) Bring (10) and (15) (a), (b), (c) to pH of 7.4 to 7.8. Filter if necessary.

Sterilization: Tube and sterilize at 10 pounds pressure for 10 minutes.

Use: To study food requirements of streptococci and pneumococci. Aminoids are an enzyme digest of milk to the amino acid stage. Author reported no growth in medium (15b).

Reference: Mueller (1922 p. 331).

836. Mueller's Heart Infusion Peptone Solutions

Constituents:

1. Water (tap)	500.0 cc.
2. Heart, beef	1.0 lb.
3. Peptone (Difco)	10.0 g.
4. NaCl	10.0 g.
5. $MgSO_4$	0.4 g.
6. $CaCl_2$	0.2 g.
7. K_2HPO_4	2.0 g.
8. Glucose	2.0 g.
9. Phenol red (0.02% soln.)	80.0 cc.

Preparation:

(1) Prepare beef heart infusion by heating 1 pound of chopped beef heart with 500.0 cc. of water to the boiling point. Strain and filter.

(2) Decolorize by boiling for 25 minutes with 10.0% "Norit" a commercial grade of wood charcoal.

(3) Filter thru paper.

(4) Make up one of the following media:

(a)	Decolorized infusion	25.0 cc.
	Glucose salt solution	25.0 cc.
	Peptone (Difco)	0.0 or 0.5 g.
(b)	Infusion (not decolorized)	25.0 cc.
	Glucose salt solution	25.0 cc.
	Peptone	0.0 or 0.5 g.
(c)	Water	25.0 cc.
	Glucose salt solution	25.0 cc.
	Peptone	0.5 g.

(5) Bring each lot to pH = 7.4 to 7.8.

(6) Filter if necessary.

(7) Tube.

Sterilization: Sterilize at 10 pounds pressure for ten minutes.

Use: To study food requirements of streptococci and pneumococci. Author reported that no growth in (3) (a) occurred without peptone, no growth in (3) (c). All others gave growth.

Reference: Mueller (1922 p. 325).

837. Huntoon's Hormone Heart Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Heart, beef	500.0 g.
3. Peptone (Bacto)	10.0 g.
4. Gelatin	10.0 g.
5. Salt	5.0 g.
6. Whole egg	1
7. Glucose	1.5 g.
8. Laked blood	

Preparation:

(1) Mix 10.0 g. Bacto peptone, 10.0 g. gelatin, 5.0 g. NaCl, one whole egg and 500.0 g. of finely chopped beef heart in a liter of water. Place in an enamel ware vessel or a large coffee pot.

(2) Heat over a free flame with constant stirring until the red color of the meat infusion changes to brown at a temperature of about 68°C. Do not go beyond this temperature.

(3) Adjust to slightly alkaline to litmus with N/1 NaOH and then add 1.0 cc. per liter of medium.

(4) Cover the vessel and place in an Arnold sterilizer or in a water bath at 100° for one hour.

(5) Remove the vessel from the sterilizer and separate with a glass rod the firm clot which has formed from the side of the vessel.

(6) Return to the Arnold sterilizer at 100° for 1½ hours.

(7) Remove the vessel and allow to stand at room temperature for about 10 minutes in a slightly inclined position.

(8) Pipette off the fluid portion or decant. If it is poured thru a fine wire sieve, many of the fine pieces of meat clot may be caught. (Avoid filtering thru cheese cloth, cotton or other adsorptive materials.)

(9) Allow (8) to stand in tall cylinders for 15 to 20 minutes until the fat present has risen to the surface and removed.

(10) The medium may be further cleared by filtering thru glass wool, asbestos wool, sedimentation or centrifugation.

(11) Add 0.15% dextrose and enough laked blood to give a slight pink tint.

(12) Tube in 10.0 cc. lots.

Sterilization: Sterilize by the intermittent method.

Use: To cultivate highly pathogenic organisms. Author reported that if spinal fluid be taken from a meningitis case in which no organism can be found in smears, a distinct growth appeared in this medium after 9 hours rendering a positive diagnosis possible. Organisms multiplied in the leukocytes transferred.

Variants:

(a) Author used fresh beef steak instead of heart.

(b) Bailey prepared a similar medium as follows:

(1) Dissolve 10.0 g. gelatin in 1000.0 cc. distilled water and heat to 50 to 60°C.

(2) Add 500.0 g. of moderately fine chopped beef to (1).

(3) Bring to a boil and cook slowly for 15 to 20 minutes.

(4) Filter thru a 16 mesh (cullender type) until clear.

(5) Add 10.0 g. peptone and 5.0 g. NaCl.

(6) Boil 5 minutes.

(7) Adjust to the desired reaction (pH = 7.5).

(8) Allow to stand several minutes and decant the supernatant fluid.

(9) Tube.

(10) Sterilize fractionally or at 5 pounds pressure for 5 minutes.

Reference: Huntoon (1918 p. 172). Bailey (1925 p. 341).

838. Macnoughton's Blood Infusion Broth

Constituents:

1. Heart infusion broth.
2. Blood.

Preparation:

(1) Prepare infusion broth using ox heart, and adjust to pH between 7.4 and 7.5.

(2) Tube in 10.0 cc. lots.

(3) The evening before the medium is required, add 1.0 cc. of sterile human blood to each 10.0 cc. of (2).

(4) Thoroughly mix by rolling the tubes between the hands.

(5) Allow to stand at room temperature over night.

Sterilization: Not specified.

Use: Isolation of gonococci.

Reference: Macnoughton (1923 p. 297).

839. Ficker and Hoffmann's Caffeine Infusion Broth

Constituents:

1. Distilled water	4000.0 cc.
2. Beef	1000.0 g.
3. Peptone (Witte) (6.2%)	120.0 g.
4. NaCl (0.5%)	10.0 g.
5. Caffein (0.6%)	24.0 g.
6. Crystal violet (0.1%) (solution)	28.0 cc.

Preparation:

(1) Pour 2 liters of distilled water over 1000.0 g. finely chopped lean beef in an enamel kettle.

(2) Weigh the kettle and contents.

(3) Heat at 50 to 55°C. for 30 minutes.

(4) Stir with a glass rod and heat to boiling.

(5) Weigh and add distilled water to make up the loss in weight.

(6) Filter thru filter gauze.

(7) Measure and add 6.0% (not given as 0.6%) Witte peptone and 0.5% NaCl.

(8) Heat until the peptone is dissolved.

(9) Filter.

(10) Distribute in Erlenmeyer or beer flasks.

(11) Provide each flask with an absorbent paper cap.

(12) Prepare a 1.2% caffeine solution in sterile distilled water. Shake, but heating is not necessary to obtain complete solution. Weigh the caffeine on a chemical balance.

(13) Measure 100.0 cc. of sterile (11) into a sterile Erlenmeyer flask and adjust to −2.7 to phenolphthalein.

(14) Measure 105 cc. of (12) in a sterile measuring flask and add to each 100.0 cc. of cool (13) under aseptic conditions. Do not heat after the addition of caffeine.

(15) Prepare a 0.1% crystal violet solution (use chemical balance) in sterile distilled cold water.

(16) Add 1.4 cc. of (15) by means of a sterile pipette to each flask (14).

Sterilization: Sterilize (1) in streaming steam for 2 hours. Sterilize (13) in streaming steam for 10 minutes.

Use: Diagnosis of typhoid. Enrichment medium for typhoid. Emulsions were made from the patients' feces and smeared, following 13 hours' incubation, on this medium.

Variants:

(a) Lubenau used 0.3% caffeine instead of 0.6% and plated on solid medium. (See medium 1749.)

(b) Abel gave the following method of preparation:

(1) Chop 500.0 g. of fat free meat and add to a liter of water at 50°C.

(2) Keep at 50°C. for 30 minutes and boil for 30 to 45 minutes.

(3) Filter or strain the fluid from the meat.

(4) Make up the fluid to one liter.

(5) Add sufficient NaOH so that the reaction is alkaline to phenolphthalein.

(6) Sterilize for 10 minutes in the steamer.

(7) Add 1050.0 cc. of a 1.2% caffeine solution and 14.0 cc. of a 0.1% crystal violet solution (both the caffeine and dye are dissolved in sterile cold water).

(8) Add the stools directly to this medium.

(c) Harvey used infusion broth prepared according to variant (bb) 779, adjusted to permanently alkaline to phenolphthalein and mixed with equal parts of a 1.0% caffeine solution. No crystal violet was employed.

References: Ficker and Hoffmann (1904 p. 255), Lubenau (1907 p. 248), Abel (1912 p. 131), Harvey (1921–22 p. 92), Klimmer (1923 p. 216).

840. Ayers and Rupp's Hippurate Infusion Broth

Constituents:

1. Meat infusion	1000.0 cc.
2. Peptone (Park Davis)	10.0 g.
3. Sodium hippurate	10.0 g.
4. Glucose	2.0 g.
5. K_2HPO_4	1.5 g.

Preparation:

(1) Method of preparation of meat infusion not given.

(2) Dissolve 2, 3, 4 and 5 in (1).

(3) Adjust the reaction to pH = 7.2.

Sterilization: Method not given.

Use: To show hydrolysis of sodium hippurate by haemolytic streptococci. When more glucose was added, and phosphate omitted, allowing the hydrogen ion concentration to increase, the streptococci continued to hydrolyse the sodium hippurate. The development of acidity had little effect.

Variants: The authors used 2.0 to 5.0 g. glucose with or without 10.0 g. of K_2HPO_4.

Reference: Ayers and Rupp (1922 p. 391).

841. MacConkey's Bile Salt Infusion Broth (Heinemann)

Constituents:

1. Infusion broth	100.0 cc.
2. Sodium taurocholate (0.5%)	0.5 g.
3. Peptone (2.0%)	2.0 g.
4. Glucose (0.5%)	0.5 g.
5. Litmus.	

Preparation:

(1) Prepare infusion broth.

(2) Dissolve 2, 3 and 4 in (1) by heating.

(3) Filter.

(4) Add sufficient litmus solution to color.

Sterilization: Method not given.

Use: Culture medium used in water analysis.

Reference: Heinemann (1905 p. 129).

841a. Smith's Glucose Infusion Broth

Constituents:

Infusion broth	1000.0 cc.
Peptone, Witte	20.0 g.
NaCl	5.0 g.
Glucose	1.0 g.

Preparation:

(1) Prepare infusion from beef in the usual manner.

(2) Add Na_2CO_3, normal solution, to adjust to 1.5 to 2.0% acid.

(3) Heat to 40°C. and inoculate with 30–50.0 cc. of a 12 to 14 hour bouillon culture of *B. coli* and incubate for 16 hours or over night.

(4) Mix with the white of egg, one egg per liter.

(5) Boil in a water bath or in an Arnold sterilizer for 45 to 60 minutes. Cool and add 2.0% Witte peptone,

and 0.5% NaCl. Dissolve by gentle heat.
(7) Add N/1 Na_2CO_3 until 0.8% acidity is reached.
(8) Boil or steam 20 or 30 minutes.
(9) Filter.
(10) Distribute into Fernbach flasks in layers 2.5 ctm. deep. Each flask should have two or three cotton-plugged openings.
(11) Add 0.1% glucose to each sterile flask of (10) from a sterile 20.0% glucose solution.

Sterilization: Sterilize (10) by heating in the autoclave at 110° to 115° for 30 minutes. Method of sterilization of glucose solution not specified.

Use: Cultivation of *B. diphtheriae* and toxin production. Author reported that the medium may be regarded as having the maximum toxicity when reaction is distinctly alkaline to phenolphthalein. Other investigators used a similar medium for different purposes.

Variants:

(a) Migula prepared a medium as follows:
  (1) Mix 500.0 g. of finely chopped lean beef with one liter of water and allow to stand in the ice box for 12 to 24 hours.
  (2) Press the liquid thru a towel and make up the volume to 1 liter.
  (3) Boil in the steam cooker for 30 minutes.
  (4) The infusion may be boiled for an hour before removing the meat and then filtered thru paper. If the liquid is still red, boil again for 15 minutes.
  (5) Filter whey cold to remove the fat.
  (6) Add 10.0 g. Witte peptone and 5.0 g. NaCl.
  (7) Neutralize by the addition of a concentrated solution of Na_2CO_3 until litmus is colored violet.
  (8) Add the desired amount of soda. Generally, 10 cc. of 15.0% soda solution is added per liter.
  (9) Boil and filter.
  (10) Add 1.0 or 2.0% glucose.
  (11) Distribute in tubes or flask.
  (12) Boil for one hour to sterilize.

(b) Copeland and Boynton used a medium prepared as follows to study the Voges-Proskauer reaction by the colon group.
  (1) Prepare an infusion from fresh beef steak.
  (2) Dissolve 10.0 g. Witte's peptone, 5.0 g. NaCl and 10.0 g. of water-free glucose in (1).
  (3) Adjust to 1.0% acid to phenolphthalein.
  (4) Sterilization not specified.

(c) Avery and Cullen prepared a medium as follows:
  (1) Prepare a beef infusion.
  (2) Dissolve 10.0 g. peptone, 10.0 g. glucose and 5.0 g. NaCl in (1).
  (3) Adjust to pH = 7.6 to 7.8.
  (4) Tube and sterilize.

  They reported that in this medium the bovine type of *Streptococcus haemolyticus* gave a final pH of 4.3 to 4.5 while a human type gave a final pH of 5.0 to 5.3.

References: Smith (1899 p. 375), Migula (1901 p. 19), Copeland and Boynton (1905 p. 242), Avery and Cullen (1919 p. 218).

842. Mueller's Meat Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. NaCl	10.0 g.
3. $MgSO_4$	0.4 g.
4. $CaCl_2$	0.2 g.
5. K_2HPO_4	2.0 g.
6. Glucose	2.0 g.
7. Phenol red (0.02% soln.)	80.0 cc.
8. Meat infusion	1000.0 cc.
9. Peptone	20.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Mix equal parts (1) and meat infusion, and add 1.0% peptone. (The author added no peptone.)
(3) Adjust to pH = 7.8. (No peptone medium pH = 7.6.)

Sterilization: Sterilize in autoclave at 10 pounds for 10 minutes.

Use: To study the food requirements for the growth of streptococci and pneumococci. The author reported that in the above medium the peptone had little influence on the growth. Using the media given under variants, all media not containing peptone gave poor growth.

Variants: The author gave the following method of preparing different media:

(1) Soak one pound of meat with 500.0 cc. of cold water, heat to 55° for a few minutes and strain. Boil this extract to remove coagulable materials and filter. This is Extract I.
(2) Take meat from extract I, add 500.0 cc. H_2O, boil 5 minutes, strain and filter. This is Extract II.
(3) Take meat from extract II, add 500.0 cc. of H_2O, boil 5 minutes, strain and filter. This is Extact III.
(4) Divide each extract into two equal parts.
(5) Mix each of these two parts with an equal amount of glucose salt solution (see step (1) in the medium).
(6) Add 2.0% peptone to one of these parts, and make no addition to the other.
(7) Adjust to pH = 7.8 when containing peptone and pH = 8.0 when not containing peptone.

Reference: Mueller (1922 pp. 316, 320, 321).

843. Robinson and Rettger's Glucose Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Lean beef	1.0 lb.
3. Peptone (Witte)	20.0 g.
4. Glucose	1.0 g.

Preparation:

(1) Extract 1.0 lb. of chopped lean beef in one liter of water in the cold for 24 hours.
(2) Coagulate on a boiling water bath.
(3) Filter and add 2.0% Witte's peptone to the filtrate.
(4) Heat on the water bath for about 20 minutes.
(5) Adjust the reaction to +0.6 to phenolphthalein.
(6) Filter and distribute into 150.0 cc. Erlenmeyer flasks in 20.0 cc. lots.
(7) After sterilization add enough sterile glucose to each flask to make 0.1% glucose.

Sterilization: Sterilize at 12 to 14 pounds pressure for 15 minutes.

Use: Cultivation of *B. diphtheriae* and toxin production.

Variants: Authors adjusted the reaction to +1.0 to phenolphthalein instead of +0.6, M.L.D. = 0.005 cc.

Reference: Robinson and Rettger (1917 p. 363)

844. Bronstein and Grünblatt's Indicator Glucose Infusion Broth

Constituents:

1. Meat infusion broth	1000.0 cc.
2. Glucose (0.5%)	5.0 g.
3. Indigo carmine solution.	
4. Fuchsin solution (acid).	

Preparation:

(1) Prepare a neutral 0.5% glucose meat infusion broth.
(2) Tube in 5.0 cc. lots.
(3) Prepare a 2.0% watery solution of indigo carmine.
(4) Dissolve 10.0 g. of acid fuchsin in 100.0 cc. of 1.0% KOH solution.
(5) Mix 2 parts (3) with 1 part (4) and add to 22 parts distilled water.

Sterilization: Not specified.

Use: Differentiation of diphtheria organisms. After 24 hours incubation add 3 drops of (5) to each tube. The authors reported that the control tube was blue; diphtheria cultures were ruby-red; pseudo diphtheria cultures were green after several minutes.

Reference: Kolle and Wasserman (1912 p. 414).

845. Ayers, Rupp and Johnson's Glucose Infusion Broth

Constituents:

1. Meat infusion	1000.0 cc.
2. Peptone (Park Davis & Co.)	10.0 g.
3. Glucose	5.0 g.
4. Sodium ricinolate (Castor oil soap).	

Preparation:

(1) Prepare 1000.0 cc. of meat infusion.
(2) Add 2 and 3 to (1).
(3) Adjust reaction to pH = 7.5.
(4) Add castor oil soap (sodium ricinolate) or other surface reducing material in sufficient amounts to lower the surface tension to about 50 and 43 dynes.

Sterilization: Method not given.

Use: To study effect of surface tension on growth of streptococci. Authors reported that in general when the surface tension was reduced to from 40 to 41 dynes the growth of streptococci was prevented.

Reference: Ayers, Rupp and Johnson (1923 p. 204).

846. Ayers, Rupp and Mudge's Glucose Infusion Broth

Constituents:

1. Meat infusion	1000.0 cc.
2. Peptone (Bacto)	10.0 g.
3. K_2HPO_4	10.0 g.
4. Glucose	10.0 g.

Preparation:

(1) Prepare 1000.0 cc. meat infusion.
(2) Add 2, 3 and 4 to (1).
(3) Adjust to pH = 7.5.
(4) Tube in 15 cc. lots in Eldredge tubes.

Sterilization: Method not given.

Use: To study fermentation of glucose by streptococci.

Reference: Ayers, Rupp and Mudge (1921 p. 258).

847. Torrey's Brilliant Green Infusion Broth

Constituents:

1. Meat infusion broth	1000.0 cc.
2. Glucose	10.0 g.
3. Brilliant green (1.0% aq. soln.)	15.0 cc.

Preparation:

(1) Prepare meat infusion broth in the usual manner.
(2) Neutralize to phenolphthalein. The final reaction must be neutral.
(3) Dissolve 10.0 g. glucose in (2).
(4) Tube in exactly 10.0 cc. quantities.
(5) Prepare a 1.0% solution of Grübler's brilliant green in distilled water.
(6) Add 0.15 cc. of (5) to each sterile tube of (4).

Sterilization: Sterilize in the Arnold steamer. Time not specified.

Use: Enrichment of the paratyphoid-enteritidis group. Author reported that other dominant fecal bacteria were inhibited by the dye.

Reference: Torrey (1913 p. 265).

848. Torrey's Acetic Acid Infusion Broth

Constituents:

1. Meat infusion broth	1000.0 cc.
2. Glucose	10.0 g.
3. Acetic acid.	

Preparation:

(1) Prepare meat infusion broth.
(2) Add 10.0 g. glucose to 1.
(3) Add acetic acid to (2) until the reaction is 0.2, 0.1 or 0.05 normal acetic acid, using phenolphthalein as an indicator.
(4) Tube in 10.0 cc. lots.
(5) Plug with paraffined cotton.

Sterilization: Method not given.

Use: To study fecal flora.

Reference: Torrey (1915 p. 74).

849. Sherman's Lactose Infusion Broth

Constituents:

1. Beef infusion	1000.0 cc.
2. Peptone	30.0 g.
3. Na_2HPO_4	10.0 g.
4. KH_2PO_4	5.0 g.
5. Lactose	20.0 g.

Preparation:

(1) Prepare 1000.0 cc. beef infusion.
(2) Dissolve 2, 3, 4 and 5 in (1).

Sterilization: Method not specified.

Use: Show CO_2 production in highly buffered medium by *Streptococcus kefir*, Author reported that in this buffered solution CO_2 production approached that in digested milk.

Reference: Sherman (1921 p. 130).

850. Harvey's Starch Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.
3. NaCl	10.0 g.
4. Starch (Corn flour)	10.0 g.
5. Peptone	10.0 g.
6. Litmus solution.	

Preparation:

(1) Mince finely fat-free beef.

Note: Veal, chicken, ox or horse heart, horse flesh, rabbit flesh, fish, blood, placenta, liver, spleen, kidneys, brain and vegetable materials, such as yeast, wheat, etc., may serve to furnish the extract used as basis for medium.

(2) Add 500.0 g. to 1000.0 cc. distilled water or clear tap water.
(3) Heat the mixture 20 minutes over a free flame, at a temperature not exceeding 50°C.

Note: Or simply keep in a cool place over night.

(4) Skim off fat floating on the surface.
(5) Raise the temperature to boiling point.
(6) Boil 10 minutes.
(7) Pour the mixture on to a wet, thick, clean cloth.
(8) Add sodium chloride 5.0 g. to the filtrate.
(9) Steam 45 minutes.
(10) Bring the volume up to 1000.0 cc. by the addition of water.
(11) Estimate and adjust the reaction.
(12) Steam for 30 minutes.
(13) Filter while hot thru well-wetted, thick filter paper.
(14) Make a paste of 10.0 g. of corn flour starch and 200.0 cc. of (13).
(15) Heat 20 minutes at 20 pounds pressure.
(16) Add 10.0 g. peptone, 5.0 g. NaCl and 800.0 cc. of (13) to (15).
(17) Steam 45 minutes.
(18) Make the reaction 0.2% acid to phenolphthalein.
(19) Steam 30 minutes.
(20) Filter while hot thru well-wetted, thick filter paper.
(21) Add saturated litmus solution to make a deep blue color.
(22) Distribute into test tubes.

Sterilization: Sterilize at 100°C. for 20 minutes on 3 days.

Use: Cultivation of meningococci.

Reference: Harvey (1921–22 p. 112).

851. Loeffler's Malachite Green Infusion Broth

Constituents:

1. Beef infusion	1000.0 cc.
2. Peptone (2.0%)	20.0 g.
3. Lactose (5.0%)	50.0 g.
4. Dextrose (1.0%)	10.0 g.
5. Sodium sulfate (0.5%)	5.0 g.
6. KNO_3 (2.0%)	20.0 g.
7. KNO_2 (1.0%)	10.0 g.
8. Malachite green 2% solution	30.0 cc.

Preparation:
(1) Prepare a beef infusion from one pound of beef.
(2) Dissolve 2, 3,t, 5, 6 and 7 in (1).
(3) Add 3.0% of 2.0% malachite green solution to (2).

Sterilization: Sterilize on each of 3 successive days for 10 minutes in streaming steam.

Use: Enrichment of the colon-typhoid group.

Variants: Klimmer prepared the medium as follows:
(1) Dissolve 2, 3, 4, 5, 6 and 7 in meat infusion broth.
(2) Flask in 100.0 cc. quantities.
(3) Sterilize on each of 3 successive days for 10 minutes in streaming steam.
(4) Add 3.0 cc. of a 2.0% solution of 120 Höchst malachite green to each 100.0 cc. lot of sterile (2).
(5) Tube in 3.0 or 4.0 cc. lots in sterile tubes.

References: Loeffler (1906 pp. 289, 295), Klimmer (1923 p. 213).

852. Duval and Lewis' Inulin Bouillon

Constituents:

1. Meat infusion broth	1000.0 cc.
2. Inulin	10.0 g.

Preparation:
(1) Prepare boiullon according to standard methods from beef. (Method or reference not given.)
(2) Adjust from 0.2 to 0.4% normal to phenolphthalein. After sterilization reaction from 0.5 to 0.8%.
(3) Distribute into clean tubes in 9.0 cc. lots.
(4) Prepare a 10.0% inulin solution in distilled water.
(5) Add 1.0 cc. of sterile (4) to each 9.0 cc. of sterile (3).

Sterilization: Sterilize (3) method not given. Sterilize (4) in the autoclave at 15 pounds pressure for 15 minutes.

Use: Cultivation of pneumococci.

Reference: Duval and Lewis (1905 p. 484).

853. Bulir's Mannitol Infusion Broth

Constituents:

1. Water	2000.0 cc.
2. Beef	1000.0 g.
3. Peptone (Witte)	25.0 g.
4. NaCl	15.0 g.
5. Mannite	30.0 g.
6. Neutral red.	

Preparation:
(1) Macerate 1000.0 g. finely chopped lean beef with 2000.0 cc. water for 24 hours.
(2) Filter thru linen, and press the meat free from water.
(3) To the liter of meat infusion thus

obtained add 25.0 g. Witte's peptone, 15.0 g. NaCl and 30.0 g. mannite.

(4) Boil for 1.5 hours over a flame.

(5) Neutralize by the addition of soda.

(6) Filter.

(7) Dissolve 0.1 g. neutral red in 100.0 g. distilled water.

(8) When ready for use add 3.0 cc. of sterile (7) to each 150.0 cc. of the medium.

(9) Distribute in 15 to 20.0 cc. lots in fermentation tubes.

Sterilization: Sterilize (6) in streaming steam. Sterilize (7) method not given.

Use: Detection of *Bacterium coli* in water. When desired for use, add 100.0 cc. of the water being investigated to 50.0 cc. of sterile (6). Then add 3.0 cc. of neutral red solution as indicated in step (8). The author reported that after 12 to 24 hours incubation at 46°C. that the presence of *Bact. coli* was indicated by a uniform turbidity in the fermentation tube, the formation of gas, and a change in color from a red to a yellow-greenish fluorescence.

Variants: Klimmer added 25.0 g. Witte's peptone, 15.0 g. NaCl and 30.0 g. mannitol in a liter of meat infusion, boiled 1.5 hours, neutralized, tubed and sterilized in streaming steam for 2 hours. The addition of neutral red or test water not specified.

References: Bulir (1907 p. 11), Klimmer (1923 p. 216).

854. Harvey's Ferric Tartrate Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Ferric tartrate	1.0 g.

Preparation:

(1) Prepare infusion broth according to Harvey's method, see variant (bb) 665.

(2) Add 1.0 g. of ferric tartrate to a liter of (1).

Sterilization: Not specified.

Use: General culture medium.

Reference: Harvey (1921–22 p. 107).

855. Harvey's Lead Acetate Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. Beef	500.0 g.
4. NaCl	5.0 g.
5. Lead acetate	1.0 g.

Preparation:

(1) Prepare meat infusion broth according to Harvey's method, see variant (bb) 665.

(2) Add 1.0 g. of lead acetate to (1).

Sterilization: Not specified.

Use: General culture medium.

Reference: Harvey (1921–22 p. 107).

856. Omeliansky's Formate Infusion Broth

Constituents:

1. Meat infusion broth	1000.0 cc.
2. Sodium formate	5.0 g.
3. Phenolphthalein	drops

Preparation:

(1) Prepare meat infusion broth from meat and peptone.

(2) Add 0.5% sodium formate to (1).

(3) Add several drops of phenolphthalein.

Sterilization: Not specified.

Use: Differentiation of colon typhoid group. Author reported that *Bact. typhi abdom.* did not decompose the formate. *Bact. coli commune* decomposed the formate with the production of gas. *Bact. paratyphi A and B* decomposed the formate with production gas. *Bact. dysenteriae* and *Bac. faecalis alcaligenes* did not decompose the formate. Some of the organisms not producing gas may turn the phenolphthalein red due to the decomposition of some albuminous material to amino acids or other compounds.

Variants: The author omitted the phenolphthalein.

Reference: Omeliansky (1905 p. 674, 1906–07 p. 158).

857. Jordan's Phenol Infusion Broth

Constituents:

1. Infusion broth	900.0 cc.
2. Phenol (1.0% soln.)	100.0 cc.

Preparation:

(1) Prepare infusion broth according to standard methods (see committee A. P. H. A. 1899, 665).

(2) Neutralize to phenolphthalein and then add 5.5 cc. of normal acid per liter.
(3) Distribute (1) in 9.0 cc. lots in test tubes.
(4) Add 1.0 cc. of a 1.0% aqueous solution of carbolic acid.

Sterilization: Sterilize in the Arnold sterilizer (time not given).

Use: Enrichment medium in water analysis.

Variants:
(a) Harvey added 1.0 to 5.0 g. of phenol to infusion broth prepared according to Harvey (see variant (bb) 665).
(b) Harvey added 4.0 cc. of strong HCl to 100.0 cc. of 5.0% phenol solution and added 0.1, 0.2 or 0.3 cc. of this mixture to 10.0 cc. of sterile meat infusion (see 665).

Reference: Jordan (1901 p. 298), Harvey (1921–22 p. 90).

858. Harvey's Egg Infusion Broth

Constituents:

1. Water	2000.0 cc.
2. Beef	500.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Whites of	4 eggs
6. Yolks of	2 eggs
7. NaOH (normal)	12.0 cc.

Preparation:
(1) Prepare meat infusion broth according to Harvey's method (see variant (bb) 665).
(2) Prepare an egg stock solution by mixing the whites of 4 eggs, the yolks of 2 eggs and 12.0 cc. normal NaOH.
(3) Add 1000.0 cc. of water to (2).
(4) Heat very slowly to 90°C.
(5) Distribute in flasks.
(6) Mix equal parts of (5) and sterile (1) when ready for use.

Sterilization: Sterilization of (1) in the autoclave or steamer. Sterilize (5) in the autoclave.

Use: General culture medium.

Variant: Harvey used a similar egg medium by preparing the egg stock solution as indicated above, or by mixing the whites of 4 eggs and 8.0 cc. of normal NaOH with 660.0 cc. of water and then diluting to a liter. When ready for use add 1 part of the egg stock solution, prepared as indicated above, to 5 parts meat infusion broth.

Reference: Harvey (1921–22 p. 86).

859. Olitsky and Kligler's Egg White Infusion Broth

Constituents:

1. Distilled water	90.0 cc.
2. Meat infusion broth	300.0 cc.
3. Egg white	10.0 cc.

Preparation:
(1) Prepare plain meat infusion broth (method not given).
(2) Add one volume of egg white to 9 volumes of distilled water.
(3) Mix 300.0 cc. of (1) and 100.0 cc. of (2), ($\frac{1}{3}$ of (2) by volume).
(4) Adjust to pH$^{=}$ from 7.6 to 7.8.
(5) Distribute in 500.0 cc. lots into 2 liter flasks to permit sufficient aeration.

Sterilization: Autoclave for 45 minutes at 15 pounds pressure.

Use: Cultivation of *Bacillus dysenteriae* Shiga, and study of toxin production. After 5 days incubation at 37° C. filter thru Berkefeld N candle. This bacteria free filtrate constitutes the exotoxin.

Variants: Dopter and Sacquépée gave the following method of preparation:
(1) Place the whites of two eggs in a glass graduate.
(2) Shake constantly, adding little by little three times their volume of distilled water.
(3) Add 2.0 cc. of 10.0% soda solution (0.5 cc. per 100.0 cc.). Mix well.
(4) Heat in the autoclave at 115°C.
(5) Prepare meat infusion peptone solution (500.0 g. beef, 5.0 g. NaCl and 20.0 g. peptone per liter).
(6) Mix one part (4) with 5 parts (5).
(7) Distribute.
(8) Sterilize for 15 minutes at 112°C.

860. Weiss and Wilkes-Weiss Egg "Hormone" Broth

Constituents

1. Hormone broth	400.0 cc.
2. Whole egg	100.0 cc.

Preparation: (1) Mix whole egg and "hormone" broth in the ratio of 1:4.

Sterilization: Heat for 30 minutes in the Arnold sterilizer.

Use: Cultivation of *Spirochaeta pallida.* The authors reported that the value of the medium would be greatly improved if a small quantity of agar be added (0.2%).

Reference: Weiss and Wilkes-Weiss (1924 p. 226).

861. Kohman's Brain Infusion Medium

Constituents:

1. Meat infusion broth
2. Brain.

Preparation: (1) Grind beef brain and add sufficient meat infusion broth to cover.

Sterilization: Sterilize in the autoclave.

Use: To cultivate meningococci and to determine the effect of oxygen tension on growth.

Reference: Kohman (1919 p. 577).

862. Kreidler's Glucose Brain Broth

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.
3. Peptone (Difco)	10.0 g.
4. NaCl	5.0 g.
5. Glucose (1.0%)	10.0 g.
6. Brain.	

Preparation:

(1) Infuse 500.0 g. of finely chopped lean beef in 1000.0 cc. water in an ice box over night.
(2) Boil 30 minutes over a free flame.
(3) Filter through gauze and then through paper to remove the fat.
(4) Make up to 1000.0 cc. volume.
(5) Add 10.0 g. peptone (Difco) and 5.0 g. NaCl.
(6) Boil to dissolve.
(7) Adjust to pH = 7.8.
(8) Filter through paper.
(9) Add 10.0 g. glucose.
(10) Fill long tubes and add several pieces of calf brain, one cubic centimeter in size, washed in running water.

Sterilization: Autoclave at 12 pounds pressure for 20 minutes.

Use: To study the bacteriology of endocarditis.

Reference: Kreidler (1926 p. 190).

863. Kligler's Heart Infusion Solutions

Constituents:

1. Meat infusion broth.
2. Saline solution.
3. Heart (beef).

Preparation:

(1) Chop beef heart into small pieces.
(2) Divide (1) into six equal portions.
(3) Take up each portion of (2) with 9 volumes of saline solution.
(4) Treat each one of the suspensions in one of the following ways:
    (a) Keep in the ice box over night and steam in the Arnold sterilizer for an hour.
    (b) Keep in the ice box over night and filter thru a Berkefeld candle.
    (c) Keep at 55°C. over night and heat in the Arnold sterilizer for an hour.
    (d) Keep at 55°C. over night and filter thru a Berkefeld candle.
    (e) Extract by boiling one hour and then steam for an hour in the Arnold.
    (f) Extract by boiling for one hour.
(5) Take 1.0 cc. of one of (4) and add to sterile meat infusion broth.

Sterilization: Method not given.

Use: To study the effect of growth stimulating materials on the growth of pathogenic bacteria. Author reported that heat destroyed the growth stimulating materials.

Reference: Kligler (1919 p. 43).

864. Havens and Taylor's Kidney and Blood Infusion Broth

Constituents:

1. Meat infusion	1000.0 cc.
2. Peptone (1.0%)	10.0 g.
3. Na_2HPO_4 (1.0%)	10.0 g.
4. Glucose (0.5%)	5.0 g.
5. Kidney (rabbit).	
6. Blood, defibrinated, (sheep or rabbit).	

Preparation:

(1) Add 2, 3 and 4 to 1000.0 cc. of ordinary meat infusion made with distilled water.
(2) Adjust to pH 8.0 to 8.2.
(3) Tube in 10.0 cc. quantities.
(4) Add a fragment of sterile rabbit kidney and 1.0 cc. of defibrinated sheep or rabbit blood to each tube of sterile (3).

Sterilization: Sterilize (3) in the autoclave at 10 pounds pressure for 10 minutes.

Use: Cultivation of hemolytic streptococci.

Reference: Havens and Taylor (1921 p. 313).

865. Robertson's Cooked Meat Medium (Torrey)

Constituents:

1. Water	1000.0 cc.
2. Beef heart	500.0 g.
3. Peptone	10.0 g.

Preparation:

(1) Mix 500.0 g. finely minced fresh beef heart with 10.0 g. peptone and a liter of water.
(2) Cook in a double boiler with just enough heat to cause a slight simmering for about 10 minutes.
(3) Adjust to pH = 7.2.
(4) Continue to cook for 1½ hours.
(5) Readjust the reaction if necessary.
(6) Decant the broth.
(7) Place the meat in test tubes to a height of 5 cm.
(8) Add 5.0 cc. of sterile broth to each sterile tube of (7).

Sterilization: Sterilize flasks of (6) and tubes of (7) separately in the autoclave at 15 pounds pressure for 15 minutes. Following mixing, place the tubes in the Arnold sterilizer on each of two successive days for 20 minutes.

Use: Analysis of fecal flora.

Reference: Torrey (1926 p. 355).

866. Wade and Manalang's Blood Infusion Broth

Constituents:

1. Distilled water.
2. Meat infusion broth.
3. Blood.

Preparation:

(1) The beef infusion broth contains Witte's peptone and NaCl in various concentrations. Exact amounts not given.
(2) Thoroly lake sheep or horse blood by adding 20.0 cc. blood to 100.0 cc. distilled water.
(3) Heat to 80 to 85°C.
(4) Precipitate the proteins while hot by adding strong hydrochloric acid.
(5) Filter first thru gauze, then thru paper.
(6) Reduce the filtrate to 1.0% acid.
(7) Mix equal parts of sterile (6) and sterile (1).

Sterilization: Method of sterilization of (1) not given. Sterilize (6) by repeated heating at 65°C. or by filtering.

Use: Cultivation of *Bacillus influenzae*, The author reported that better growth was obtained using double strength broth.

Variants: Author mixed (6) with equal parts of double strength broth.

Reference: Wade and Manalang (1920 p. 98).

867. Harvey's Ascitic Fluid Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. Beef	500.0 g.
4. NaCl	5.0 g.
5. Ascitic fluid	500.0 cc.

Preparation:

(1) Prepare meat infusion broth according to Harvey's method, (see variant (bb) 665).
(2) Mix 2 parts (1) with one part ascitic fluid.
(3) Test sterility before use by incubation 48 hours.

Sterilization: Sterilize in the water bath 30 minutes at 56°C., on each of five successive days.

Use: Cultivation of parasitic and saphrophytic bacteria.

Reference: Harvey (1921–22 p. 83).

868. Stryker's Serum Infusion Broth

Constituents:

1. Meat infusion broth	900.0 cc.
2. Serum	100.0 cc.

Preparation:

(1) Prepare meat infusion broth.
(2) Mix 1 part highly potent anti-pneumococcus horse serum with 9 parts normal horse serum.
(3) Mix 100.0 cc. of (2) with 900.0 cc. of (1).

Sterilization: Method not specified.

Use: To study variation in pneumococcus. Other investigators used similar media for different purposes.

Variants:

(a) The author used plain serum instead

of a mixture of immune and plain serum.

(b) Lucke and Rea cultivated streptococci on a similar medium. They reported that typical streptococci gave a heavy white sediment consisting of albumin. Their medium was prepared as follows:
  (1) Prepare a meat infusion broth (2.0% peptone).
  (2) Boil 1000.0 cc. of (1) to 80.0% of its original volume and adjust to 0.4+.
  (3) Tube and sterilize for 3 days in steam.
  (4) Obtain horse serum aseptically and heat to 60°C. for 1 hour in a water bath to destroy antihemolysins.
  (5) Add 2.0 cc. of (4) to each 8.0 cc. of (3).

(c) Stevens, Brady and West studied the relation between virulence and hemolysin. They reported that a strain of streptococcus whose virulence had been increased did not produce greater concentrations of hemolysin. Their medium was prepared as follows:
  (1) Prepare a meat infusion broth with 2.0% peptone.
  (2) Titrate (1) so that it is pH = 7.6 after sterilization (method not mentioned).
  (3) Distribute into 250.0 cc. Pyrex flasks in 80.0 cc. lots.
  (4) Add 20.0 cc. of fresh horse serum to each flask.
  (5) Inactivate the contents at 56°C. on 3 successive days, and store on ice until ready for use.

(d) Harvey mixed 2 parts serum with 1 part infusion broth, (see variant (bb) 665). The mixture was sterilized for 60 minutes at 57°C. on each of 2 successive days.

References: Stryker (1916 p. 50), Lucke and Rea (1919 p. 535), Stevens (1921 p. 224), Harvey (1921–22 p. 82).

869. Foster's Serum Infusion Broth

Constituents:

1. Beef infusion broth	1000.0 cc.
2. Glucose	10.0 g.
3. Horse serum broth	5.0 g.

Preparation:
  (1) Prepare beef infusion broth.
  (2) Adjust (1) to pH = 6.3.
  (3) Prepare (2) 10.0% glucose solution.
  (4) Mix (1) and (2).
  (5) Add sterile 3 to sterile (4) in sufficient quantity to give a 1.0% concentration of glucose.

Sterilization: Sterilize (3) method not given. Sterilize (4) at 15 pounds pressure for 15 minutes.

Use: To study protein and carbohydrate metabolism of *Streptococcus hemolyticus*. General culture medium.

Variants: Harvey cultivated meningococci on a medium containing 1 part unheated clear sterile horse serum to 20 parts infusion broth (see variant (bb) 770) to which was added 1.0% glucose.

References: Foster (1921 p. 222), Harvey (1921–22 p. 80).

870. Beach and Hasting's Serum Infusion and Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Lean beef	450.0 g.
3. Peptone (Difco)	15.0 g.
4. K_2HPO_4	7.5 g.
5. Liebig's beef extract	4.0 g.
6. Glycerol	55.0 cc.
7. Aminoid peptone from beef (Arlco)	5.0 g.
8. Serum (sterile blood)	100.0 cc.

Preparation:
  (1) Extract 450.0 g. lean beef with 1000.0 cc. water for 3 hours. Bring the water to 45°C. After an hour raise the temperature to 50°C. and during the third hour to 55°C. Stir frequently.
  (2) Remove the major portion of the liquid from the meat.
  (3) Heat meat and remaining liquid to 100°C. This causes the meat particles to shrink giving the maximum amount of liquid.
  (4) To the liquid (2) plus that of (3) add 10.0 g. peptone, 5.0 g. K_2HPO_4, 2.0 g. Liebig's beef extract and 50.0 cc. of glycerol.
  (5) Adjust the reaction to pH of 7.5 to 7.8.

(6) Distribute in 100.0 cc. lots in 500.0 cc. Erlenmeyer flasks.
(7) Seed half the sterilized flasks with the grass bacillus of Karlensky and the remainder with the glass bacillus of Moeller.
(8) Incubate at 37.5°C. for two weeks.
(9) Then heat to 100°C.
(10) Cultures are then filtered and filtrate made up to 1000.0 cc.
(11) Add 5.0 g. peptone, 2.5 g. K_2HPO_4, 2.0 g. Liebig's beef extract, 50.0 cc. glycerol and 5.0 g. of aminoid peptone from beef (Arlco).
(12) The reaction is left unchanged.
(13) Filter and distribute 50.0 cc. lots in 500.0 cc. Erlenmeyer flasks.
(14) Add 5.0 cc. of sterile horse blood serum (serum must be raw) just before inoculation.

Sterilization: Sterilize (6) and (13) separately in the autoclave, time not given.

Use: Preparation of johnin. Johnin is a material used for detection of Johnin's disease, much as tuberculin is used for tuberculosis. The medium was inoculated heavily with the organism causing Johnin's disease. Cover the flasks with tin foil to prevent evaporation and incubate 10 to 16 weeks.

Reference: Beach and Hastings (1922 p. 72).

871. Harvey's Blood Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.
3. NaCl	14.0 g.
4. $CaCl_2$	0.25 g.
5. KCl	0.42 g.
6. Glucose	25.0 g.
7. Blood, citrated plasma	200.0 cc.

Preparation:
(1) Prepare meat infusion solution as indicated in medium 729.
(2) Dissolve 3, 4, 5 and 6 in 1.
(3) Tube in 5.0 cc. quantities.
(4) Add 1.0 cc. of citrated humas plasma to each tube of (3).

Sterilization: Method not given.

Use: Cultivation of gonococci.

Reference: Harvey (1921–22 p. 82).

872. Park, Williams and Krumwiede's Potato Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Meat infusion	1000.0 cc.
3. Potato	1.0 lb.
4. Peptone	20.0 g.
5. NaCl	10.0 g.

Preparation:
(1) Grate 1.0 pound of white potatoes or run thru a chopping machine.
(2) Soak in a liter of water over night.
(3) Heat to boiling.
(4) Press thru cheese cloth.
(5) Add one egg per liter.
(6) Autoclave one hour to clarify.
(7) Filter thru cotton (very tedious).
(8) Flask.
(9) Soak 1 pound of finely chopped lean beef in a liter of tap water over night in the ice box or at room temperature.
(10) Weigh (9) and heat at 45°C. for 1 hour and then boil for 30 minutes.
(11) Make up the loss in weight by the addition of hot water.
(12) Strain thru cheese cloth and squeeze by twisting the cloth or use a meat press.
(13) Mix equal parts of (8) and the juice from (12).
(14) Dissolve 20.0 g. peptone and 10.0 g. NaCl in (13).
(15) Distribute as desired.

Sterilization: Autoclave for 30 minutes at 15 pounds pressure.

Use: General culture medium.

Reference: Park, Williams and Krumwiede (1924 p. 122).

873. Lubinski's Potato Infusion Broth

Constituents:

1. Water	1500.0 cc.
2. Potato	1000.0 g.
3. Meat	500.0 g.
4. Peptone	10.0 g.
5. NaCl	5.0 g.
6. Glycerol	40.0 g.

Preparation:
(1) Wash potatoes clean and cut them in small pieces.
(2) Boil for 3 or 4 hours under a free flame or in a steamer.

(3) Filter.
(4) To one liter of (3) add 500.0 g. finely chopped meat.
(5) Allow to stand for 24 hours.
(6) Strain.
(7) Add and dissolve 4 and 5 in (6).
(8) Boil to precipitate albuminous materials.
(9) Filter.
(10) Add 4.0% glycerin.
(11) Neutralize (may be used without neutralization).

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

Reference: Lubinski (1895 p. 126).

874. Jurewitsch's Potato Infusion Broth

Constituents:

1. Water	1500.0 to 2000.0 cc.
2. Potato	500.0 g.
3. Meat	500.0 g.
4. Peptone (Witte or Chapoteaut)	0.5%
5. NaCl	0.25%
6. Glycerol	3.0%

Preparation:

(1) Peeled potatoes are cut into large pieces, washed off with water and pulverized.
(2) Weigh the potato pulp and add an equal weight or double weight of water depending on the type of potato. If the potato contains lots of juice do not add so much water.
(3) Allow (2) to infuse in the cold for one day, shake and strain thru a straining cloth.
(4) After ¼ to ½ hour pour the liquid from the sediment.
(5) During this time prepare a meat infusion using 500.0 g. of finely chopped meat to 1000.0 g. of water. Press thru a straining cloth after 24 hours.
(6) Mix equal parts of (5) and (4).
(7) Add 0.5% Chapoteaut's or Witte's peptone and 0.25% NaCl.
(8) Warm gently until peptone is completely dissolved.
(9) Boil in a Koch steamer for 1 hour and filter warm thru a folded filter. It may be necessary to allow the mixture to settle and pour off the clear liquid and filter that.
(10) Add 3.0% glycerin and add a saturated solution of sodium carbonate until the reaction is decidedly alkaline.
(11) Boil in the autoclave for ¼ to ½ hour at 118–120°C., cool and filter.
(12) Distribute as desired.

Sterilization: Sterilize at 115°C. for ½ to 1 hour.

Use: Cultivation of tubercle bacilli. Medium should be dark brown in color.

Variants:

(a) The author used 0.25% HK_2PO_4 instead of NaCl.
(b) Kolle and Wesserman gave the following method of preparation:
    (1) Prepare a pulp from clean peeled potatoes.
    (2) Add 1000.0 cc. of water to 500.0 g. of (1) and allow to stand for one day in the cold.
    (3) Shake thoroughly and filter through linen.
    (4) Allow to settle for 15 to 30 minutes and decant the clear water.
    (5) Soak 500.0 g. of lean meat in 1000.0 cc. of water for 24 hours.
    (6) Press thru linen.
    (7) Mix equal parts of (4) and (6).
    (8) Add 1% peptone and 0.5% NaCl and boil for one hour.
    (9) Filter.
    (10) Add 3% glycerol and make distinctly alkaline by the addition of a saturated soda solution.
    (11) Autoclave for 15 to 30 minutes.
    (12) Filter.
    (13) Sterilize once more.

Reference: Jurewitsch (1908 p. 664), Kolle and Wasserman (1912 p. 409).

875. Olitsky and Gates' Bacteria Infusion Broth

Constituents:

1. Meat infusion broth	1000.0 cc.
2. Glucose	10.0 g.
3. *B. coli communis*	

Preparation:

(1) Prepare meat infusion broth with 1.0% peptone.
(2) Add 1.0% dextrose to (1).
(3) Distribute in 100.0 cc. lots.
(4) Seed with a broth culture of *B. coli communis*.

(5) Incubate until a faint haze is formed (usually 1 to 2 hours).
(6) Cover with a layer of sterile paraffin.

Sterilization: Steam (6) at 100° for 15 to 30 minutes.

Use: Cultivation of *Bacteriun pneumosintes*. The medium was inoculated while the layer of paraffin was still liquid.

Reference: Olitsky and Gates (1922 p. 818).

876. Ogata's Porphyra Infusion Broth

Constituents:

1. Meat infusion broth	1000.0 cc.
2. Glucose	25.0 g.
3. *Porphyra vulgaria*	50.0 g.

Preparation:

(1) Prepare meat infusion, using 500.0 g. of meat per liter.
(2) Dissolve 2 and 3 in (1).
(3) Boil.
(4) Neutralize.
(5) Filter.
(6) Tube.

Sterilization: Method not given.

Use: Isolation and cultivation of protozoa (infusoria). For isolation a capillary tube is filled with the medium, inoculated by placing one end of the tube into the culture and the tube sealed.

Reference: Ogata (1893 p. 168).

877. Park, Williams and Krumwiede's Stomach Digest Infusion Broth

Constituents:

1. Water	2000.0 cc.
2. Meat	1.0 lb.
3. Peptone (1.0%)	10.0 g.
4. NaCl (0.5%)	5.0 g.
5. Stomach (hog)	200.0 g.

Preparation:

(1) Clean 5 pigs' stomachs, remove the fat and mince finely. (A number of stomachs should be used to equalize the peptone content. In this way an almost average composition in peptone is obtained.)
(2) Mix 200.0 g. of (1), 10.0 g. HCl, pure, and 1000.0 cc. of water at 50°C.
(3) Incubate at 50°C. for 20 to 24 hours in a glass or porcelain vessel, not enamel. It is most important not to allow the digest to come in contact with any metal until neutralized.
(4) Heat to 80°C. to stop digestion.
(5) Pass thru a layer of absorbent cotton.
(6) The digest may be stored without sterilization.
(7) Heat the filtrate from (5) to 70°C.
(8) Neutralize to litmus at 70°C.
(9) Mix an equal volume of (5) and infusion broth, prepared according to Park, Williams and Krumwiede (see variant (ii) medium 779).
(10) Adjust to the desired reaction at room temperature.
(11) Autoclave for 15 minutes to clear.
(12) Readjust the reaction as necessary.
(13) Filter thru paper and cotton to clear.

Sterilization: Sterilize in the autoclave at 15 pounds pressure for 30 minutes.

Use: General culture medium.

Reference: Park, Williams and Krumwiede (1924 p. 118).

878. Hitchen's Yeast Autolysate Blood Solution

Constituents:

1. Beef infusion.	
2. Blood clot	200.0 cc.
3. Peptone (1.0%)	10.0 g.
4. NaCl (0.5%)	5.0 g.
5. Agar (0.07%)	0.7 g.
6. Glucose (3.0%)	30.0 g.
7. Maltose (3.0%)	30.0 g.
8. Yeast.	

Preparation:

(1) Dissolve 200.0 cc. of strained horse blood clot, 10.0 g. peptone, 5.0 g. NaCl and 0.7 g. agar in sufficient beef infusion to make a liter.
(2) Adjust the reaction to pH 5.4.
(3) Autoclave and filter while hot.
(4) Distribute in 500.0 cc. lots in liter flasks.
(5) After sterilization, add sufficient sterile solutions of glucose and maltose to make a 3.0% solution of each of the sugars.
(6) Inoculate with yeast and keep at room temperature until ready for use. (One week is sufficient.)
(7) Place the yeast cultures at 55°C. over night and then bring rapidly to the boiling point over a free flame.
(8) Heat at 95°C. for 15 minutes.
(9) The autolysate may be filtered thru

a mandler or allow to sediment and pour off the supernatant fluid.

(10) Add 75.0 cc. of (9) to flasks containing 1500.0 cc. of beef infusion.

Sterilization: Final sterilization not specified.

Use: Cultivation of influenza bacilli, pneumococci and streptococci.

Reference: Hitchens (1922 p. 35).

879. Hitchens' Semisolid Glucose Agar (Mulsow)

Constituents:

1. Water	1000.0 cc.
2. Beef	1.0 pound
3. Peptone	20.0 g.
4. KNO$_3$	2.0 g.
5. Agar (0.2%)	1.0 g.
6. Glucose	2.0 g.

Preparation:

(1) Add 500.0 cc. of water to 1 pound of ground lean beef, and allow to stand at 37°C. for 48 hours.

(2) Express the juice and add 20.0 g. peptone, 2.0 g. KNO$_3$ and an equal amount (500.0 cc.) of a 0.2% agar solution heated and cooled to 60°C.

(3) Adjust the reaction to +0.9 to phenolphthalein.

(4) Heat in the autoclave at 15 pounds pressure for 25 minutes.

(5) Filter and readjust the reaction if necessary.

(6) Add 2.0 g. glucose.

Sterilization: Final sterilization not specified.

Use: Cultivation of gonococcus. Mulsow reported that the gonococcus grew as well if not better than any contaminating organism during the first 12–18 hours.

Reference: Mulsow (1925 p. 423).

880. Menten and Manning's Lactose Bile Infusion Solution

Constituents:

1. Infusion broth	100.0 cc.
2. Bile, fresh ox	900.0 cc.
3. Peptone	10.0 g.
4. Lactose	10.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Enrichment of enteritidis-paratyphoid B organisms from animal tissue.

Reference: Menten and Manning (1925 p. 401).

881. Hitchen's Glucose Agar Infusion Solution (Mulsow)

Constituents:

1. Water	1000.0 cc.
2. Beef	1.0 lb.
3. Peptone	20.0 g.
4. KNO$_3$	2.0 g.
5. Agar	1.0 g.
6. Glucose	2.0 g.

Preparation:

(1) Grind one pound of lean beef and infuse in 500.0 cc. of water at 37°C. for 48 hours.

(2) Add 20.0 g. peptone, 2.0 g. KNO$_3$ and an equal amount of an 0.2% agar solution, heated and cooled to 60°C. to the juice from (1).

(3) Adjust to +0.9 to phenolphthalein.

(4) Heat in the autoclave at 15 pounds pressure for 25 minutes.

(5) Filter and adjust the reaction if necessary.

(6) Add 2.0 g. glucose.

(7) Final reaction to be between 6.6 and 6.8 with an optimum at pH 6.8.

Sterilization: Final sterilization not specified.

Use: Isolation of gonococci. The author reported that the gonococci grew as well if not better than the contaminating organism the first 12 to 18 hours. The addition of one part ascitic fluid to four parts agar gave only slightly better results.

Reference: Mulsow (1925 p. 420).

882. Kreidler's Trypsinized Broth

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.
3. Peptone (Difco)	10.0 g.
4. NaCl	5.0 g.
5. Trypsin (10% soln.)	10.0 cc.

Preparation:

(1) Infuse 500.0 g. finely chopped lean beef in 1000.0 cc. water in an ice box over night.

(2) Boil 30 minutes over a free flame.

(3) Filter thru gauze and then thru paper to remove the fat.

(4) Make up to 1000.0 cc. volume.

(5) Add 10.0 g. peptone (Difco) and 5.0 g. NaCl.

(6) Boil to dissolve.

(7) Adjust to pH = 7.8.

(8) Filter thru paper.
(9) To 1000.0 cc. of sterile (8) add 10.0 cc. of a sterile 10% solution of trypsin in normal NaCl solution.

Sterilization: Sterilize (8) by the fractional method in the Arnold. Sterilize the trypsin solution by passing through a porcelain filter.

Use: To study the bacteriology of endocarditis.

Reference: Kreidler (1926 p. 190).

883. Richardson's Carcinoma Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Tumor mass	500.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Glucose	20.0 g.
6. Tartaric acid	10.0 g.

Preparation:

(1) Remove the tumor mass from the breast and strip off as much fat as possible. A few pieces of muscle clear of all fat are also included.
(2) Weigh this material, chop finely and make up in the same proportions as in ordinary bouillon. (Exact proportions not given.)
(3) Boil thoroly, filter and boil again.
(4) While in boiling condition add peptone and salt as in ordinary bouillon. (Exact amounts not given.)
(5) Neutralize carefully (method not given), boil again for a short while and while hot add 2.0% glucose and 1.0% tartaric acid.
(6) Cool in a flask—boil again and filter and tube hot.

Sterilization: Sterilize in the Arnold sterilizer 3 times, one-half hour each time. When a tube is to be inoculated, sterilize for ½ hour just before inoculation.

Reference: Richardson (1900–01 p. 72).

884. Siebert's Horse Meat Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Horse meat	250.0 g.
3. Peptone (Witte's)	10.0 g.
4. NaCl	5.0 g.
5. Glycerol	37.0 g.

Preparation:

(1) Prepare an infusion from 1 part horse meat and 4 parts water.
(2) Dissolve 3, 4 and 5 in (1).
(3) Adjust so that there is 10.0 cc. of N/1 alkali per liter using litmus as an indicator.
(4) Distribute in 40.0 cc. lots in Erlenmeyer flasks.

Sterilization: Method not given.

Use: Cultivation of tubercle bacilli.

Reference: Siebert (1909 p. 305).

885. Peklo's Potato Horse Meat Infusion Broth

Constituents:

1. Potato juice	500.0 cc.
2. Meat (horse infusion)	500.0 cc.
3. Peptone (Witte's)	5.0 g.
4. KH_2PO_4	2.5 g.
5. Glycerol	30.0 g.

Preparation:

(1) Prepare a pulp from raw potatoes.
(2) Add a little distilled water.
(3) Allow to stand in the cold for 24 hours.
(4) Press thru a towel.
(5) Heat until no more coagulum is formed.
(6) Filter.
(7) Prepare a meat infusion from horse meat in the same manner as in steps (1) thru (6).
(8) Mix 500.0 cc. of (6) and (7).
(9) Dissolve 3, 4 and 5 in (8).
(10) Add Na_2CO_3 so that the reaction will be alkaline after sterilization.

Sterilization: Method not given.

Use: Cultivation of plant actinomyces.

Variants: The author gave the following variants:

(a) Omitted the K_2HPO_4 and glycerol.
(b) Omitted the glycerol, used 5.0 g. K_2HPO_4 instead of 2.5 g. and added 1.0 g. K_2CO_3, and 1.5 g. $MgSO_4$. The medium was filtered and sterilized.

Reference: Peklo (1910 p. 551).

886. Hida's Horse Meat Infusion Broth

Constituents:

1. Water	1000.0 cc.
2. Meat, horse	500.0 g.
3. Burdock root	40.0 g.

4. Peptone (Witte)	20.0 g.
5. NaCl	5.0 g.

Preparation:

(1) Boil 500.0 g. finely chopped horse meat and 40.0 g. of finely chopped burdock root in 1 liter of water for two hours.
(2) Filter.
(3) Add 20.0 g. Witte's peptone and 5.0 g. NaCl to the bluish filtrate.
(4) Neutralize by the addition of alkali, using phenolphthalein, or an indicator.
(5) Add 6.0 cc. of a normal alkali solution to (4).
(6) Boil for another hour.
(7) Cool and filter.
(8) Distribute in flasks.

Sterilization: Sterilize in the autoclave.

Use: Cultivation of diphtheria bacilli and toxin production. Author reported that 0.001 cc. of a 10 day culture at 33°C. killed a 250.0 g. guinea pig in 4 days. The roots of the compositae generally contain from 2 to 3% inulin.

Variants: The author substituted *Arctium lappa compositae* for burdock roots.

Reference: Hida (1910 p. 412).

887. Khouvine's Fecal Infusion Broth

Constituents:

1. Water	750.0 cc.
2. Fecal infusion	250.0 cc.
3. Cellulose	250.0 cc.
4. $CaCO_3$	2.0 g.
5. Peptone (pancreatic)	1.0 g.
6. NaCl	1.0 g.
7. K_2HPO_4	1.0 g.

Preparation:

(1) Mix one part feces with 8 to 10 parts distilled water.
(2) Filter on a Laurent filter until clear.
(3) Allow to settle and decant the clear liquid.
(4) Mix 250.0 cc. of sterile (4) with 750.0 g. of water and 250.0 cc. of precipitated cellulose that has been dissolved in Schweitzer's reagent.
(5) Dissolve 4, 5, 6 and 7 in (4).

Sterilization: Sterilize the filtrate from (2) in the autoclave at 110°C. for 15 minutes. Sterilization of other material or final sterilization not specified.

Use: Isolation and enrichment of *B. cellulose dissolvens.*

Reference: Khouvine (1923 p. 715).

888. Cutler's Blood Clot Infusion Broth (Stitt)

Constituents:

1. Water	1000.0 cc.
2. Blood clot (human)	500.0 cc.
3. NaCl (0.5%)	5.0 g.
4. Peptone (1.0%)	10.0 g.
5. Blood	

Preparation:

(1) Take 500.0 cc. of human blood clot and boil for one hour in 1 liter of water.
(2) Filter.
(3) Add 1.0% peptone and 0.5% NaCl.
(4) Tube.
(5) Add a few drops of blood to each sterile tube just before use.

Sterilization: Sterilize (4) for 20 minutes on each of 3 successive days.

Use: Cultivation of *Entamobea histolytica* and other intestinal protozoa or amoeba.

References: Cutler (1918 p. 22), Stitt (1923 p. 51).

889. Percival's Urea Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Beef extract (Lemco)	50.0 g.
3. Peptone (Witte)	10.0 g.
4. Urea	100.0 g.

Preparation:

(1) Boil 2, 3 and 4 in 1.
(2) Cool.
(3) Medium should be slightly alkaline. If not, add a drop or two of $(NH_4)_2CO_3$ solution.
(4) Place in a 250.0 cc. flask.

Sterilization: Not specified.

Use: Cultivation of soil forms, *Urobacillus Pasteurii.* Medium inoculated with garden soil. Author reported that at first a stained mount showed Urococci or Planosarcins. After a few days, however, a pure culture of *Urobacillus Pasteurii* developed.

Reference: Percival (1920 p. 223).

890. Stutzer's Dinitro Benzol Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Potassium citrate	10.0 g.

3. Dinitro benzol	2.0 g.
4. K_2HPO_4	1.0 g.
5. Peptone	
6. Meat extract (Schülke and Meyer)	

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Reaction to be slightly alkaline.
(3) Distribute in 10.0 cc. lots.

Sterilization: Method not given.

Use: To study denitrification. Author reported no growth using *B. agilis*, *B. nitrovorus*, *B. Stutzeri*, *B. Hartlebi*.

Reference: Stutzer (1901 p. 86).

891. Harrison and Vanderleck's Aesculin Extract Broth

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone	10.0 g.
3. Liebig's Meat Extract	2.0 g.
4. Iron citrate	2.5 g.
5. Aesculin	1.0 g.
6. Bile salt (Commercial)	5.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Adjust reaction to +1.0.

Sterilization: Not specified.

Use: Detection of *B. coli* and *B. typhosus*. *B. coli* caused a blackening of the medium.

Reference: Harrison and Vanderleck (1909 p. 622).

892. Sawin's Glycocholate Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. Lactose	10.0 g.
4. Beef extract	7.5 g.
5. Gelatin	5.0 g.
6. Sodium glycocholate	2.0 g.
7. Pancreatin	1.0 g.

Preparation:
(1) Heat 2, 3, 4 and 5 in 1 over boiling water or in the steamer for 30 minutes.
(2) Neutralize in the usual way (method not given).
(3) Add 2.0 g. of sodium glycocholate dissolved in a little hot water and 1.0 g. pancreatin to (2).

Sterilization: Not specified.

Use: Presumptive test for *B. coli*. Enrichment medium in water analysis. Author reported that best results were obtained when pancreatin was present. *B. coli* produced gas.

Variants: The author omitted the pancreatin.

Reference: Sawin (1909 p. 388).

893. Olszewski and Köhler's Trypsinized Bile Salt Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. Liebig's meat extract	5.0 g.
4. NaCl	5.0 g.
5. Sodium taurocholate (0.1%)	1.0 g.

Preparation:
(1) Prepare ordinary nutrient bouillon using 10.0 g. peptone, 5.0 g. Liebig's meat extract and 5.0 g. NaCl.
(2) Make alkaline by the addition of 7.0 cc. N/1 soda solution after neutralizing to litmus.
(3) Boil.
(4) Cool to 40°C. and add 0.2 g. trypsin, 10.0 cc. chloroform and 5.0 cc. toluol.
(5) Place in a glass stoppered flask and place in the incubator for 24 to 48 hours, shaking occasionally.
(6) Filter thru a moistened folded filter paper.
(7) Add 0.1% sodium taurocholate to (6). (Not specified if the salt or gall is to be added before the dilution with physiological salt solution or not.)
(8) Distribute in 5.0 cc. lots.

Sterilization: Not specified.

Use: Indol production. Dilute one part of (6) with 5 parts physiological salt solution when desired for use.

Variants: Author used 2.0% sterile beef bile instead of sodium taurocholate.

Reference: Olszewski and Köhler (1923 p. 6).

894. Frieber's Tryptophane Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Peptone (1.0%)	10.0 g.
3. Meat extract (Liebig's 0.5%)	5.0 g.
4. NaCl (0.5%)	5.0 g.
5. Tryptophane (0.03%)	0.3 g.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Neutralize and add 6.0 cc. of normal soda solution per liter.
(3) Dissolve 0.03% tryptophane in (2).

Sterilization: Not specified.

Use: Indol production. Author reported that Salkowski's test (H_2SO_4 + nitrites) gave a positive test with materials other than indol. Indol was not formed in the presence of glucose.

Variants: The author added 3.0% glucose and about 10.0% sterile $CaCO_3$.

Reference: Frieber (1921–22 p. 265).

895. Bachmann's Indicator Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Dextrose (0.5%)	5.0 g.
3. Peptone (0.5%)	5.0 g.
4. Meat extract (0.5%)	5.0 g.
5. Indigo carmine (0.04%)	0.4 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Adjust the reaction to a slightly alkaline reaction by the addition of Na_2CO_3.

Sterilization: Not specified.

Use: Cultivation of obligate anaerobes. Other investigators used the medium for various purposes.

Variants: Rogers, Clark and Davis tested the reduction ability of lactic acid organisms using the following solution:

1. Water	1000.0 cc.
2. Beef extract	4.0 g.
3. Peptone	10.0 g.
4. Glucose	5.0 g.
5. Neutral red (Grübler's, 0.5% soln.)	10.0 cc.

References: Bachmann (1912–13 p. 7), Rogers, Clark and Davis (1914 p. 415).

896. Rogers, Clark and Evans' Glucose Extract Broth

Constituents:

1. Distilled water	1000.0 cc.
2. Beef extract (Liebig)	4.0 g.
3. Peptone (Witte)	10.0 g.
4. Dextrose (Kahlbaum)	10.0 g.
5. K_2HPO_4	5.0 g.

Preparation:

(1) Add 2 and 3 to 1, heat on steam bath for 20 minutes.

(2) Filter and make up loss of water.

(3) Neutralize with N/1 NaOH using phenolphthalein.

(4) Heat 5 minutes, filter.

(5) Test reaction and neutralize again and filter if necessary.

(6) Dissolve 4 and 5 in (5).

(7) Make up (6) to 1000.0 cc.

Sterilization: Method not given.

Use: To study fermentation and buffer effect.

References: Rogers, Clark and Evans (1914 p. 103), Burton and Rettger (1917 p. 165).

897. Waksman and Joffe's Glucose Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.
4. Liebig's meat extract	5.0 g.
5. Glucose	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Adjust reaction to pH = 8.0.

(3) Tube in 10–12 cc. lots.

Sterilization: Sterilize at 15 pounds pressure for 15 minutes.

Use: To study changein reaction by actinomycetes metabolism.

Reference: Waksman and Joffe (1920 p. 41).

898. Whipple's Glucose Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Beef extract (Liebig's)	10.0 g.
3. Peptone	10.0 g.
4. Dextrose	10.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Adjust the reaction to 0.

(3) Tube in Smith fermentation tubes.

Sterilization: Sterilize in the autoclave.

Use: Presumptive test for *B. coli*. Author reported that *B. coli* produced 25 to 70% gas in the closed arm. Different investigators used similar media for various purposes.

Variants:

(a) Henneberg cultivated lactic acid bacteria in the same medium using 5.0% glucose instead of 1.0%.

(b) Heinze studied the nitrogen assimilation of green algae, *Chlorella*, *Chlorothecium* and *Stichococcus*, and reported that nitrogen was not assimilated. He used a medium containing 0.5% meat extract, 0.5% peptone and 0.5% glucose per liter of water.

(c) Buchan studied the ability of organisms found in ice cream to give the

Voges-Proskauer reaction and used the following medium:

1. Distilled water	980.0 cc.
2. Glucose	5.0 g.
3. Peptone	10.0 g.
4. Lemco	5.0 g.

(d) Zikes cultivated, cladothrix, *Cladothrix dichotoma* and sphaerotilus in the following medium:

1. Distilled water	1000.0 cc.
2. Peptone	2.5 g.
3. Glucose	2.5 g.
4. Meat extract	0.5 g.

References: Whipple (1902 p. 424), Henneberg (1903 p. 7), Heinze (1906 p. 647), Buchan (1910 p. 108), Zikes (1915 p. 533).

899. Johnson's Glucose Phenol Extract Broth

Constituents:

1. Distilled water	1100.0 cc.
2. Beef extract (Liebig's)	10.0 g.
3. Peptone	10.0 g.
4. Glucose	10.0 g.
5. Phenol	5.0 cc.
6. HCl	4.0 cc.

Preparation:

(1) Dissolve 2, 3 and 4 in a liter of distilled water.

(2) Adjust to neutral to phenolphthalein.

(3) Dissolve 5.0 g. of phenol and 4.0 cc. of HCl in 100.0 cc. of water. (This is Parietti's solution.)

(4) Add 20.0 cc. of (3) to (2).

Sterilization: Method not given.

Use: Isolation of *Bacillus coli communis* from alimentary tract of fish.

Reference: Johnson (1905 p. 351).

900. Weisser's Sucrose Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Sucrose (1.0%)	10.0 g.
3. Peptone (0.1%)	1.0 g.
4. Meat extract (0.1%)	1.0 g.
5. Litmus—to color	

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Make slightly alkaline by the addition of soda.

(3) Add litmus until the solution is colored light blue.

(4) Distribute in Erlenmeyer flasks.

Sterilization: Not specified.

Use: Cultivation of cholera and typhoid group. Other investigators used similar media for different purposes.

Variants:

(a) Author used 5.0%, 10.0% or 20.0% sucrose instead of 1.0% and added 1.0% normal NaOH solution.

(b) Author used 20.0% sucrose and added 2.0% normal NaOH solution.

(c) Author used 10.0% sucrose, 1.0% peptone, 0.5% meat extract, omitted the litmus and added 2.0% normal NaOH solution.

Reference: Weisser (1886 pp. 335, 339).

901. Koegel's Lactose Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. Lactose	10.0 g.
4. Meat extract (Liebig's)	5.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Method not given.

Use: Cultivation of *L. bulgaricus.* Other investigators have used similar media for a variety of purposes.

Variants:

(a) Obst used a medium containing 1.0% peptone, 1.0% lactose and 0.5% Liebig's meat extract for the presumptive test for *B. coli* in water analysis. She reported that generally this gave a more delicate test for gas production than a medium containing bile.

(b) Ritter used 3.0 g. Liebig's beef extract, 10.0% peptone and 10.0 g. lactose per liter for the presumptive test for *B. coli* in water analysis.

(c) Gompertz and Vorkaus used a medium containing 4.0 g. Liebig's meat extract, 10.0 g. Merck's peptone and 5.0 g. NaCl, and 50.0 g. lactose per liter to cultivate *B. acidophilus* for therapeutical purposes. The authors reported that an incubation of 60 hours gave about one hundred million bacilli to each cc. of clear culture. The organisms were transferred to a sterile 8 ounce bottle and kept until desired for therapeutic purposes.

(d) Winslow and Dolloff studied the effect of certain triphenyl-methane dyes on the growth of the colon bacilli. He added 10.0% lactose to a solution of 8.0 g. of Difco dehydrated nutrient broth and added the dyes from a 1.0% stock solution. He reported that

Rosolic acid in dilution of 1 to 1000 inhibited.

Gentian violet in dilution of 1 to 5000 to 50,000 inhibited.

Brilliant green in dilution of 1 to 10,000 to 1,000,000 inhibited.

References: Koegel (1914 p. 460), Obst (1916 p. 76), Ritter (1919 p. 610), Gompertz and Vorhaus (1921 p. 498), Winslow and Dolloff (1922 p. 303).

902. Gottheil's Sucrose Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Meat extract (Liebig's)	10.0 g.
3. Peptone	10.0 g.
4. Sucrose	10.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Method not given.

Use: Cultivation of organisms found in the soil, on roots and on rhizomes.

Variants: The author gave the following variants:

(a) Added 0.02% tartaric acid.

(b) Used 10.0 g. peptone, 10.0 g. Liebig's meat extract and 5.0 g. of sucrose to 1200.0 cc. of water and added 5.0 g. glucose, 5.0 g. lactose and 1.0 g. of Seignette salt (Rochelle salt).

(c) von Wahl used the medium with only 1.0 g. sucrose instead of 10.0 g. and variant (b) to cultivate the carrot bacillus (*Bacillus daucarum*) and other organisms causing vegetable spoilage.

References: Gottheil (1901 p. 432), von Wahl (1906 p. 495).

903. Gordon's Starch Extract Broth

Constituents:

1. Distilled water	1000.0 cc.
2. Lemco	10.0 g.
3. Peptone	10.0 g.
4. Starch	5.0 g.
5. Litmus	to color
6. $NaHCO_3$	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Tinge with litmus.

Sterilization: Not specified.

Use: Determine fermentation of starch by *V. cholerae asiaticae.* Author reported that *V. cholerae asiaticae* produced acid within 24 hours. *Vibrio Finkler* and *Prior* gave only a feeble acid reaction after 3 days. Staphylococci, streptococci, *B. diphtheriae*, *B. coli*, *B. enteritides* (Gärtner), *B. typhosus*, *B. proteus*, all failed to ferment starch with the production of acid.

Reference: Gordon (1906 p. 5).

904. La Garde's Dextrin Peptone Solution

Constituents:

1. Water	1000.0 cc.
2. Peptone	5.0 g.
3. Dextrin	5.0 g.
4. Meat extract	trace

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of *Phycomyces nitens*, *Mucor mucedo*, *Mucor Rouxii*, *Mucor corymbifer*, *Mucor spinosus*, *Mucor rasemosus*, *Mucor rhizopodiformis*, *Mucor stolonifer.*

Reference: La Garde (1911–12 p. 248).

905. Banning's Glycerol Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Glycerin	10.0 g.
3. Peptone	10.0 g.
4. Meat extract	10.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Do not adjust the reaction.

Sterilization: Method not given.

Use: To study oxalic acid formation by *Bact. Pasteurii*, *Bact. aceti*, *Bact. xylinum*, *Bact. Dortmund*, *Bact. ascendens.* La Garde used a similar medium for the cultivation of a number of moulds.

Variant: La Garde used a solution containing 10.0 g. peptone, 5.0 g. glycerol and a trace of meat extract per liter for the cultivation of *Phycomyces nitens*, *Mucor mucedo*, *Mucor Rouxii*, *Mucor corymbifer*, *Mucor spinosus*, *Mucor race-*

mosus, Mucor rhizopodiformis, Mucor stolonifer.

References: Banning (1902 pp. 395 and 425), La Garde (1911–12 p. 248).

906. Jacobson's Mannitol Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Meat extract (Liebig's)	10.0 g.
3. Peptone (Witte's)	10.0 g.
4. NaCl	5.0 g.
5. Mannitol	10.0 g.
6. Litmus solution	50.0 cc.

Preparation:

(1) Prepare a sugar free bouillon containing 1% Liebig's meat extract, 1% peptone and 0.5% NaCl.

(2) Add about 20.0 cc. N/1 NaOH to neutralize.

(3) Boil for about one hour.

(4) Filter.

(5) Add 1% mannitol and 5.0% litmus solution.

(6) After sterilization, distribute in sterile tubes.

Sterilization: Sterilize in a steam bath.

Use: To determine fermentation of mannitol by the typhoid bacilli.

Reference: Jacobson (1910 p. 211).

907. Harrison and van Der Leck's Citrate Aesculin Extract Broth

Preparation and composition the same as for medium 891 except no bile salt is added.

908. De Gaetano's Potato Extract Broth (Kamen)

Constituents:

1. Potato infusion	1000.0 cc.
2. Meat extract (Liebig's)	20.0 g.
3. Peptone	10.0 g.
4. Dextrose	100.0 g.
5. Tartaric acid	5.0 to 10.0 g.

Preparation:

(1) Preparation of potato infusion (Kartoffelaufguss) not given.

(2) Dissolve 2, 3, 4 in 1.

(3) Filter.

(4) Distribute in 6.0 cc. lots in test tubes.

Sterilization: Method not given.

Use: Cultivation of *Saccharomyces septicus.*

Reference: De Gaetano (1897 p. 200).

909. Albus and Holm's Beef Extract Medium (Medium X)

Constituents:

1. Water	1000.0 cc.
2. Yeast (1.0%)	10.0 g.
3. Peptone (1.0%)	10.0 g.
4. Lactose (1.0%)	10.0 g.
5. Beef extract (1.0%)	10.0 g.
6. Brom cresol purple (5.0% alcoholic soln.)	0.5 cc.
7. Sodium recinolate.	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Divide in four lots, and keep one as a control. To the other three add varying quantities of sodium recinolate to lower the surface tension. Surface tension determined by weight drop method following sterilization.

Sterilization: Method not given.

Use: To determine the effect of surface tension on growth of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus.* The author reported that *L. bulgaricus* was inhibited at a surface tension lower than 40 dynes while *L. acidophilus* exhibited good growth in the same media depressed to 36 dynes.

Variants: The authors used sodium taurocholate and sodium glycocholate instead of sodium recinolate to lower the surface tension.

Reference: Albus and Holm (1926 p. 14).

910. Flint's Serum Extract Broth

Constituents:

1. Water	750.0 cc.
2. Serum, blood	250.0 cc.
3. Peptone	15.0 g.
4. Glucose	15.0 g.
5. Salt	3.0 g.
6. Meat extract	3.0 g.

Preparation:

(1) Mix 1, 2, 3, 4, 5 and 6.

(2) Make alkaline by the addition of KOH.

(3) Filter.

(4) Tube.

Sterilization: Sterilize in the Arnold.

Use: Cultivation of streptococci. Author reported that streptococci grew abundantly in 24 hours and caused a flaky precipitate filling the lower portion of the tube.

Reference: Flint (1896 p. 4).

911. Grace and Highberger's Ascitic Fluid Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Beef extract (Liebig's) (0.3%)	3.0 g.
3. Peptone (Difco) (1.0%)	10.0 g.
4. NaCl (0.5%)	5.0 g.
5. Glucose (0.2%)	2.0 g.
6. Ascitic fluid (5.0%)	50.0 cc.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add 0.2% glucose and 5.0% sterile ascitic fluid that has been made alkaline by the addition of 2.5% of a 40.0% NaOH solution.

(3) Adjust to pH = 6.4, 6.8, 7.2, 7.6 or 8.0.

(4) Heat for 15 minutes at 15 pounds pressure.

(5) Filter.

(6) Tube and sterilize.

Sterilization: Sterilize (1) in the autoclave by heating at 20 pounds pressure for 15 minutes. Sterilize the ascitic fluid by autoclaving at 15 pounds pressure for 15 minutes. Final sterilization in the autoclave.

Use: To study the effect of reaction on the growth of *Streptococcus viridans*. Author reported that best growth was obtained in the pH range 6.4 and 7.6. Growth in pure culture nearly as good in a 1.0% glucose broth omitting the ascitic fluid.

Reference: Grace and Highberger (1920 p. 451).

912. Olszewski and Köhler's Bile Extract Broth

Constituents:

1. Gall	500.0 cc.
2. Peptone (Witte)	50.0 g.
3. Meat extract (Liebig)	25.0 g.
4. NaCl	50.0 g.
5. Trypsin	1.0 g.
6. Litmus solution	600.0 cc.
7. Lactose	75.0 g.

Preparation:

(1) Boil 50.0 g. Witte's peptone, 25.0 g. Liebig's meat extract and 25.0 g. NaCl with 500.0 cc. of filtered and sterile gall.

(2) Neutralize with N/1 soda solution to litmus and then add 3.5 cc. of the normal soda solution.

(3) Cool to 40°C. and add 1.0 g. trypsin (Grübler's), 5.0 cc. chloroform and 1.0 cc. toluol.

(4) Place in a glass stoppered flask, and place in an incubator at 37°C. for 36 hours, shaking occasionally.

(5) Filter thru a double filter paper, moistened with gall.

(6) Dissolve 75.0 g. lactose and 25.0 g. NaCl in 600.0 cc. of Kubel and Tiemann's litmus solution (from Kahlbaum's litmus).

(7) Boil 15 minutes, filter and neutralize with normal soda solution.

Sterilization: Sterilize the filtrate from (5) method not given. Sterilize (7), method not given.

Use: Indol production in water analysis. Before use mix 5 parts of sterile (5) with 6 parts of sterile (7) and distribute in 10.0, 5.0, 2.5, and 1.0 cc. lots in such sized containers so that 100.0, 25.0 and 10.0 cc. of water under investigation may be added to the flasks.

Reference: Olszewski and Köhler (1923 p. 312).

913. Rosenow's Brain Bacto Broth (Haden)

Constituents:

1. Distilled water	1000.0 cc.
2. Nutrient broth (Bacto)	8.0 g.
3. NaCl	8.0 g.
4. Glucose (c.p.)	2.0 g.
5. Andrade Indicator	10.0 cc.
6. Calf brain.	
7. Marble.	

Preparation:

(1) Dissolve 2 and 3 in 1 by heating.

(2) Cool and add 4 and 5.

(3) Tube in 6 by 0.75 inch test tubes so that the depth is at least 3.5 to 4.0 inches.

(4) Add 3 pieces of crushed calf brain about 1.0 cmm. square and two or three pieces crushed marbles to each tube.

Sterilization: Autoclave for 20 minutes at 15 pounds pressure.

Use: Isolation of bacteria from infected teeth.

Reference: Haden (1923 p. 831).

914. Hoffmann and Fischer's Nutrose Extract Broth (Heinemann)

Constituents:

1. Water, tap	1000.0 cc.
2. Peptone	10.0 g.
3. Extract, meat	3.3 g.
4. Nutrose (1.0%)	10.0 g.
5. Caffein (0.5%)	5.0 g.
6. Crystal violet 1.0% solution.	

Preparation:

(1) Prepare extract broth according to Heinemann's method using 1, 2 and 3 (see medium 695).

(2) Dissolve 1.0% nutrose, 0.5% caffein and 1.0% solution of crystal violet in (1).

Sterilization: Not specified.

Use: Enrichment of colon typhoid group.

Reference: Heinemann (1905 p. 130).

915. Keim's Honey Extract Broth

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. Meat extract	10.0 g.
4. Honey	25.0 g.
5. NaCl	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Neutralize.

Sterilization: Method not given.

Use: Substitute for glucose broth.

Reference: Keim (1923 p. 604).

916. Otaki and Akimoto's Sodium Citrate Blood Extract Solution

Constituents:

1. Water	1000.0 cc.
2. Meat extract	10.0 g.
3. Peptone	10.0 g.
4. Saponin (Merck)	1.0 g.
5. Sodium citrate (neutral)	20.0 g.
6. Blood.	

Preparation:

(1) Dissolve 2 and 3 in 1 by boiling.

(2) Neutralize (1).

(3) Add 4 and 5 to (2).

(4) To 5.0 cc. of (3) add 2.0 cc. of blood.

Sterilization: Not specified in the abstract.

Use: Enrichment of typhoid from the blood. Saponin is added to lake the erythrocytes.

Reference: Otaki and Akimoto (1922 p. 101) taken from (1923 p. 331).

917. Dimitroff's Egg Sea Water Medium

Constituents:

1. Sea water (75.0%)	750.0 cc.
2. Extract broth (25.0%)	250.0 cc.
3. Egg white.	

Preparation:

(1) Place small cubes of hard boiled egg white in 10.0 cc. of beef extract broth.

(2) Mix 25.0% of sterile (1) with 75.0% sterile sea water.

Sterilization: Method not given.

Use: Cultivation of *Spirillum virginianum.* This medium supported life of the organism for a few days.

Variants:

(a) The following medium gave good results, according to the author.

Physiological salt solution	200.0 cc.
Egg cube medium (same as (1))	50.0 cc.
Liquid 2.0% agar in distilled water	30.0 cc.

(b) The author reported that the following semisolid medium gave a luxuriant growth of the organism. The spirilla grew luxuriantly in this medium even when the egg cube medium was omitted.

Liquid 2.0% agar in distilled water	40.0 cc.
Distilled water	68.0 cc.
Egg cube medium (same as (1))	20.0 cc.

Reference: Dimitroff (1926 p. 21–22).

918. Löhnis' Urea Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Urea (2.0 or 10.0%)	20.0 or 100.0 g.

Preparation: (1) Dissolve 2.0% or 10.0% urea in bouillon.

Sterilization: Sterilize in the usual manner.

Use: Demonstrate the decomposition of urea.

Variants:

(a) Geilinger sterilized the bouillon, added a sterile watery urea solution to the bouillon to obtain the 10.0% strength of urea. The urea solution

was sterilized by heating at one-half atmosphere pressure for 30 minutes.
(b) Cunningham added 2.0, 5.0 or 10.0% urea to bouillon and sterilized by the intermittent method in flowing steam.

Reference: Löhnis (1913 p. 95), Geilinger (1917 p. 246), Cunningham (1924 p. 143).

919. Baruchello's Caffein Bouillon

Constituents:

1. Water	650.0 cc.
2. Bouillon	350.0 cc.
3. Caffein	10.0 g.

Preparation:
(1) Method of preparation of bouillon not given.
(2) Mix 1, 2 and 3.
(3) Adjustment of reaction not specified.

Sterilization: Sterilize in the usual manner.

Use: Caffein hindered development of *B. coli*.

Reference: Baruchello (1905 p. 570).

920. Ficker's Crystal Violet Caffein Bouillon (Bezançon)

Constituents:

1. Meat infusion	1000.0 cc.
2. Peptone	30.0 g.
3. NaCl	2.5 g.
4. Caffein	6.0 g.
5. Crystal violet	0.0007 g.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in meat infusion.
(2) Neutralize to phenolphthalein by adding 21.0 cc. of 10 normal soda solution.

Sterilization: Not specified.

Use: Enrichment of the colon-typhoid group.

Reference: Bezançon (1920 p. 345).

921. Müller's Lactose Ferro-cyanide Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Lactose	25.0 g.
3. Potassium ferro-cyanide	30.0 cc.
4. Extrait de fer pommé	30.0 cc.

Preparation:
(1) Prepare bouillon.
(2) Neutralize (2) and add 10.0 cc. of normal NaOH per liter.
(3) Add 25.0 g. sterile lactose to sterile (2).
(4) Prepare a solution of 1 part "Extrait de fer pomme" to 100.
(5) Prepare a solution of 1 part potassium ferro-cyanide to 100.
(6) Mix 1000.0 cc. (3), 30.0 cc. of sterile (4) and 30.0 cc. of sterile (5) under aseptic conditions.
(7) Distribute as desired into sterile containers.

Sterilization: Method of sterilization of (2), lactose, (4) or (5) not given.

Use: Differentiation of colon-typhoid bacilli. Author reported that colon bacilli caused a blue precipitate to develop. Typhoid bacilli caused no change in color, a brown precipitate formed.

Reference: Müller (1922 p. 984).

922. Frost's Glucose Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Glucose (1.0%)	10.0 g.

Preparation:
(1) Prepare bouillon and add 1.0% glucose.
(2) Tube in test tubes or fermentation tubes.

Sterilization: Sterilize in the steamer.

Use: General culture medium.

Variants:
(a) Kendall, Day and Walker specified the use of a sugar free bouillon.
(b) Stitt added small pieces of $CaCO_3$ (marble) to tubes of glucose bouillon.
(c) Klimmer used a 0.5% glucose bouillon, tubed in 5.0 cc. lots. After inoculation and incubation, 3 drops of the following indicator solution was added to each tube:

1. Distilled water	22.0 cc.
2. 2.0% watery indigo	2.0 cc.
3. Carmine solution	
4. 10.0% acid fuchsin solution	1.0 cc.

Klimmer differentiated pseudo diphtheria and diphtheria bacilli in this medium. He reported uninoculated bouillon blue, diphtheria culture red and pseudo diphtheria culture green after a few minutes. Pseudo diphtheria culture red also after 36 hours.

References: Frost (1903 p. 64), Heinemann (1905 p. 127), Gage (1908 p. 282), Bornand (1913 p. 516), Kendall, Day and Walker (1914 p. 419), Stitt (1921 p. 35), Klimmer (1923 p. 223).

923. Rivas' Glucose Bouillon

Constituents:

1. Bouillon	958.0 cc.
2. Glucose	10.0 g.
3. Peptone	15.0 g.
4. Ammono-sulpho-hydrate water	40.0 cc.
5. Sodium indigo sulphuric acid (10.0% soln.)	2.0 cc.

Preparation:

(1) Prepare bouillon.

(2) Add 1.0% dextrose and 1.5% peptone.

(3) Prepare a 10.0% sodium indigo sulphuric acid solution in distilled water.

(4) Heat (3) at 100°C. for one hour.

(5) Prepare a 1.0% ammonia solution in sterile distilled water.

(6) Place 150–200.0 g. of distilled water in an Erlenmeyer flask and stopper with cotton. Place a bent rod thru the cotton reaching the bottom of the flask. Plug the other end of the rod with cotton.

(7) When cool pass a stream of washed hydrogen sulphide from a Kipp generator thru the water for 5 minutes.

(8) Prepare 10 test tubes, containing 10.0 cc. of (7) each.

(9) To a series of tubes add 1 drop, 2 drops, 3 drops, etc., of (5) to each tube. Shake thoroughly.

(10) To each tube of (9) add 3 drops of a 10.0% methylene blue solution in 50.0% alcohol.

(11) Observe in which tube a complete decolorization takes place in about 1 minute (usually required 3 to 6 drops).

(12) When the proper amount of ammonia has been determined, pour 20.0 cc. of (7) into a sterile cylinder.

(13) Add the proper amount of ammonia (usually 10 to 20 drops) to the 20.0 cc. of hydrogen sulphite water. This solution is to be kept cool or a turbidity will develop.

(14) To 958.0 cc. of sterile (2) add 21.0 cc. of (13) (20.0 cc. of H_2S water and 1.0 cc. of indigo sulphuric acid solution). The medium should become clear after 1 minute.

(15) Distribute in tubes, and cover with a layer of sterile olive oil. Plug with cotton and seal with gum. This process should be done quickly.

(16) Incubate at 37°C. for 48 hours discarding any tubes showing turbidity or any sign of color.

Sterilization: Sterilize (6) at 120°C. for 15 minutes.

Use: Cultivation of anaerobes.

Variants: The author omitted the ammono-sulpho-hydrate (steps (5) thru (14)), but prepared a 1.0% Na_2SO_3 solution and added 50.0 cc. to 948.0 cc. of glucose bouillon containing 2.0 cc. of a 1.0% solution of sodium indigo sulphuric. The medium was covered with olive oil as specified above. This medium is inferior to the one described above.

Reference: Rivas (1902 p. 836).

924. MacConkey's Iodide Glucose Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Glucose	20.0 g.
3. KI	5.0 g.

Preparation:

(1) Prepare bouillon.

(2) Dissolve 2 and 3 in (1).

(3) Tube in fermentation tubes.

Sterilization: Sterilize in a steamer for 10 minutes on each of two successive days, taking care not to over heat.

Use: Enrichment of colon-typhoid group.

Variants: Author added 10.0 or 20.0 g. KI instead of 5.0 g.

Reference: MacConkey (1905 p. 337).

925. Kitasato's Formate Glucose Bouillon (Tanner)

Constituents:

1. Bouillon	1000.0 cc.
2. Glucose	20.0 g.
3. Sodium formate	4.0 g.

Preparation:

(1) Dissolve 2 and 3 in bouillon.

(2) Tube.

Sterilization: Method not given.

Use: General culture medium.

Reference: Tanner (1919 p. 47).

926. Heymann's Acetic Acid Glucose Bouillon (Finkelstein)

Constituents:

1. Bouillon	1000.0 cc.

2. Glucose	20.0 g.
3. Acetic acid	5.0 g.

Preparation: (1) Add 2 parts glucose, and 0.5 parts acetic acid to bouillon.

Sterilization: Not specified.

Use: Enrichment of aciduric bacilli from stools. Heinemann and Hefferan isolated *B. vulgaricus*.

Variants: Heinemann and Hefferan specified the use of glacial acetic acid.

References: Finkelstein (1900 p. 263), Heinemann and Hefferan (1909 p. 308).

927. Savage's Neutral Red Glucose Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Glucose (0.5%)	5.0 g.
3. Neutral red (Grübler's 0.5% soln.)	10.0 cc.

Preparation:

(1) Add 0.5% glucose to bouillon.

(2) Prepare a 0.5% watery solution of Grübler's neutral red.

(3) Add 0.1 cc. of (3) to every 10.0 cc. of (1).

Sterilization: Method not given.

Use: Detection of *Bacillus coli* in water analysis. (Neutral red reaction.) *Bacillus coli* gave a yellow coloration to the medium.

References: Savage (1901 p. 437), Gage and Phelps (1902 p. 407), Irons (1902 p. 315).

928. DeKorti's Oleic Acid Glucose Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Oleic acid (1.0%)	10.0 g.
3. Glucose (1.0%)	10.0 g.

Preparation:

(1) Prepare ordinary bouillon.

(2) Add 1.0% oleic acid and 1.0% glucose to (1).

(3) Tube in 10.0 cc. quantities.

(4) Pour a layer of vaseline on the surface of the broth in each tube.

Sterilization: Method not given.

Use: Enrichment of acne bacilli. Medium inoculated by adding a suspension of a comedo crushed in a little normal salt solution. Seal the hole made by the pipette by means of heat. Author reported that after incubation at 37° for 4 days, large numbers of acne bacilli were found present.

Reference: De Korti (1921 p. 250).

929. Plimmer's Tartaric Acid Infusion Broth

Constituents:

1. Infusion broth	1000.0 cc.
2. Glucose	20.0 g.
3. Tartaric acid	10.0 g.

Preparation:

(1) Prepare infusion broth in the usual manner.

(2) Neutralize carefully.

(3) Dissolve 2 and 3 in (2).

Sterilization: Method not given.

Use: Enrichment of Krebs-organisms.

Reference: Plimmer (1899 p. 806).

930. Gage's Lactose Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Lactose	10.0 g.
3. Neutral red 0.5% soln.	20.0 cc.

Preparation:

(1) Exact method of preparation of bouillon not given.

(2) Adjust (1) to +0.8.

(3) Add 1.0% lactose and 2.0% neutral red (0.5% aqueous solution).

(4) Filter.

(5) Tube.

Sterilization: Method not given.

Use: Primarily for the presumptive test for *Bacillus coli* in water analysis. Author reported that *Bacillus coli* produced acid and a yellowish green fluorescence.

Variants:

(a) Koegel omitted the neutral red and used 6.0% lactose instead of 1.0%. The medium was used for the cultivation of bulgaricus.

(b) Cunningham used 1.0% of Andrades indicator and 0.5% lactose instead of neutral red and 1.0% lactose.

References: Gage (1908 p. 282), Koegel (1914 p. 460), Cunningham (1924 p. 102).

931. Hall and Ellefson's Gentian Violet Lactose Broth

Constituents:

1. Water	1000.0 cc.
2. Bouillon	1000.0 cc.
3. Lactose (1.0%)	10.0 g.
4. Gentian violet, 1:100,000 or 1:20,000	

Preparation:

(1) Prepare 2.0% lactose bouillon.

(2) Add an equal volume of water to (1) giving a 1.0% concentration of lactose.

(3) Add 1 to 100,000 or 1 to 20,000 of gentian violet.

Sterilization: Not specified.

Use: Presumptive test for *B. coli.* Author reported that dye inhibited other organisms.

References: Hall and Ellefson (1918 p. 337), Levine (1921 p. 110).

932. Jouan's Citrate Lactose Bouillon (Besson)

Constituents:

1. Water	850.0 cc.
2. Bouillon	150.0 cc.
3. Lactose	10.0 g.
4. Magnesium citrate	1.0 g.
5. Litmus.	

Preparation:

(1) Dissolve 150.0 cc. of bouillon, 10.0 g. lactose and 1.0 g. of magnesium citrate in 850.0 cc. of water.

(2) Add sufficient litmus solution to give a violet color.

(3) Filter.

(4) Tube.

Sterilization: Sterilize at 110 to 112°C.

Use: Synthetic whey.

Reference: Besson (1920 p. 208).

933. Wollman's Starch Bouillon

Constituents:

1. Water	600.0 cc.
2. Bouillon	400.0 cc.
3. Starch	30.0 g.
4. Peptone (Chapoteaut)	2.0 g.

Preparation:

(1) Prepare bouillon.

(2) Add 400.0 cc. of (1) to 600.0 cc. of water.

(3) Dissolve 3 and 4 in (2).

Sterilization: Not specified.

Use: Cultivation of *Glycobacter proteolyticus.*

Reference: Wollman (1912 p. 616).

934. Hölzel's Glycogen Bouillon

Constituents:

1. Bouillon	1000.0 to 1500.0 cc.
2. Glycogen	20.0 to 100.0 cc.

Preparation:

(1) Prepare sugar free bouillon.

(2) Add 10.0 to 15.0 cc. of (1) to 0.2 to 1.0 g. of Merck's glycogen.

(3) Cool quickly after sterilization.

Sterilization: Sterilize in the autoclave in the usual manner.

Use: Cultivation of anthrax bacilli.

Reference: Hölzel (1913 p. 149).

935. Heinemann's Glycerol Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Glycerol (6.0%)	60.0 g.

Preparation: (1) Add 6.0% glycerol to bouillon.

Sterilization: Not specified.

Use: General culture medium.

Variants:

(a) Kendall, Day and Walker specified the use of sugar free bouillon and used 30.0 g. of glycerol instead of 60.0 g. per liter.

(b) Owen also used a 3.0% glycerol bouillon to determine the amount of alcohol formed in the fermentation of glycerol by *Bacillus saccharalis.*

References: Heinemann (1905 p. 127), Kendall, Day and Walker (1914 p. 419), Owen (1916 p. 241), Dopter and Sacquépée (1921 p. 119), Stitt (1923 p. 35), Cunningham (1924 p. 165).

936. Kendall, Day and Walker's Mannitol Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Mannitol	10.0 g.

Preparation:

(1) Prepare nutrient sugar free bouillon.

(2) Dissolve 2 in 1.

(3) Distribute in 100.0 cc. lots.

Sterilization: Not specified.

Use: To study the metabolism of tubercle bacilli. Authors reported that they used alizarin, neutral red and phenolphthalein to study changes in reaction; Ziehl-Neelsen stain for staining.

Reference: Kendall, Day and Walker (1914 p. 419).

937. Giltner's Adonitol Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Adonitol (0.5%)	5.0 g.

Preparation: (1) Dissolve 0.5% adonitol in bouillon.

Sterilization: Not specified.

Use: Differentiation of colon-aerogenes group. Author reported that *B. aerogenes* and *B. cloacae* fermented adonitol.

Reference: Giltner (1921 p. 383).

938. Vincent's Phenol Bouillon

Constituents:

1. Bouillon.
2. Phenol.

Preparation:

(1) Preparation of bouillon not given.

(2) Distribute in 10.0 cc. lots in tubes.

(3) To each tube add 5 drops of a 5.0% phenol solution (1 drop of 5.0% phenol to each 2.0 cc. bouillon).

Sterilization: Not specified.

Use: Enrichment of typhoid bacilli. Water analysis. Add 5 to 10 drops of the water under investigation to each tube. Author reported that contaminated water clouded the medium. Other organisms were inhibited. To obtain a pure culture of typhoid bacilli 3 or 4 sub-cultures were made in this medium.

Variants: Kamer added 5.0 g. of phenol and 4.0 g. of pure HCl to 100.0 cc. distilled water and then added 4, 6 or 9 drops (30 drops = 60 cc.) of this mixture to 10.0 cc. of sterile bouillon contained in tubes.

References: Vincent (1890 p. 63), (1900 p. 45), Kamer (1892 p. 35).

939. Thjötta and Avery's Yeast Bouillon

Constituents:

1. Distilled water	400.0 cc.
2. Brewer's yeast	100.0 g.
3. Plain broth	

Preparation:

(1) Exact method of preparation or composition of plain broth not given.

(2) Adjust the reaction of (1) to pH = 7.8.

(3) Emulsify 100.0 g. of brewers yeast in 400.0 cc. of distilled water with the reaction of pH = 4.6.

(4) Boil over a free flame for 10 minutes.

(5) Allow to sediment at room temperature.

(6) Pipette off the clear supernatant extract and test sterility.

(7) Store in ice chest until ready for use. Readjust the reaction of the extract from pH = 7.3 to 7.5 just before use.

Sterilization: Sterilization of yeast extract effected in step (4) in the preparation. Sterilization of bouillon not specified.

Use: To study bacterial nutrition of *Bacillus influenzae*. Authors reported that the medium supported the growth of *B. influenzae*. Growth accessory materials resisted boiling for 10 minutes, were destroyed by autoclaving, would pass thru a Berkefeld filter but were absorbed by charcoal. Broth containing extract alone, however, did not give continued growth of the influenza bacilli. Primary inoculations were made from blood broth.

Reference: Thjötta and Avery (1921 p. 100).

940. Thjötta and Avery's Tomato Bouillon

Constituents:

1. Bouillon.
2. Tomatoes.

Preparation:

(1) Preparation of bouillon not given.

(2) Readjust the reaction to pH = 7.8.

(3) Sear the skin off a ripe tomato with a red hot knife.

(4) Plunge a sterile fork thru this area.

(5) Dip the tomato into alcohol and flame off, then plunge into boiling water.

(6) Remove the peeling with sterile forceps.

(7) Place the tomatoes in a sterile enamel dish and crush with a sterile pestle. (Reaction of juice pH = 4.2.)

(8) Boil the juice 10 minutes.

(9) Filter the juice thru a Berkefeld candle, or clear by centrifugation.

(10) Test sterility and store in its original acidity.

(11) Just before use readjust the reaction from pH = 7.3 to 7.5.

(12) Make dilutions of the extract in (2) in the proportion of 1:10 and 1:100.

Sterilization: As indicated in steps (8) and (9) of the preparation. Sterilization of the bouillon not specified.

Use: To study bacterial nutrition of *Bacillus influenzae*. Authors reported that the medium supported growth of *B. influenzae*. Growth accessory materials resisted boiling for 10 minutes, were destroyed by autoclaving, would pass thru a Berkefeld filter but were absorbed by charcoal. Broth containing extract alone, however, did not give continued growth of the influenza bacilli. Primary inoculations were made from blood broth.

Reference: Thjötta and Avery (1921 p. 100).

941. Thjötta Pea or Bean Bouillon

Constituents:

1. Distilled water	100.0 cc.
2. Green peas or beans	100.0 g.
3. Plain broth	

Preparation:

(1) Preparation of bouillon not given.
(2) Adjust the reaction to pH = 7.8.
(3) Flame the surface of green pea or string bean pods.
(4) Open the pods with sterile forceps and crush out the separate seeds into different sterile dishes.
(5) Add an equal weight of distilled water to the emulsion and adjust the reaction of pH = 4.6.
(6) Boil 10 minutes.
(7) Strain thru glass wool and filter thru a Berkefeld filter (N).
(8) Test sterility, and store on ice until ready for use. Readjust the reaction from pH = 7.3 to 7.5.
(9) Dilute each of the extracts in (2) in the proportions 1:10 and 1:100.

Sterilization: Sterilization of pea or bean extract effected in steps (6) and (7) in preparation. Sterilization of bouillon not given.

Use: To study bacterial nutrition of *Bacillus influenzae*. Author reported that the medium supported growth of *B. influenzae*. Growth accessory materials resisted boiling for 10 minutes, were destroyed by autoclaving, would pass thru a Berkefeld filter, but were absorbed by charcoal. Broth containing extracts alone, however, did not give continued growth of the influenza bacilli. Primary inoculations were made from blood broth.

Reference: Thjötta and Avery (1921 p. 100).

942. Thjötta and Avery's Potato Bouillon

Constituents:

1. Distilled water	400.0 cc.
2. Brewers yeast	100.0 g.
3. Plain broth	
4. Potato	

Preparation:

(1) Preparation of bouillon not given.
(2) Adjust the reaction of (1) to pH = 7.8.
(3) Emulsify 100.0 g. of brewers yeast in 400.0 cc. of distilled water with the reaction of pH = 4.6.
(4) Boil over a free flame for 10 minutes.
(5) Allow to sediment at room temperature.
(6) Pipette off the clear supernatant extract and test sterility.
(7) Store in ice chest until ready for use. Readjust the reaction of the extract from pH = 7.3 to 7.5 just before use.
(8) Procure sterile pieces of potato from the center of an old potato without abrasions or imperfections. Take all possible precautions to remove the pieces in as asterile a condition as possible.
(9) Distribute the sterile pieces of potato into (2).
(10) Add yeast extract (7) amount not given.

Sterilization: Sterilization of yeast extract effected in step (4) in the preparation. Sterilization of bouillon not given.

Use: To study bacterial nutrition of *Bacillus influenzae*. Author reported that growth in unheated potato occurred equally well without the addition of yeast extract. When the potato was heated at 120° for 45 minutes no growth occurred unless the yeast extract was added. Medium did not give continued growth. Initial inoculation made from blood broth.

Variants: The authors omitted the yeast extract.

Reference: Thjötta and Avery (1921 p. 109).

943. Thjötta's Bacterial Emulsion Bouillon

Constituents:

1. Bouillon.
2. Bacterial emulsion.

Preparation:

(1) Prepare bouillon with a reaction of pH = 7.8.
(2) Grow bacteria (mucoid bacillus (Friedländer's), *Bacillus ozoenoe* and

B. proteus were organisms used) on plain agar slants.

(3) Suspend the growth of the organism in a few drops or 1.0 cc. of sterile plain bouillon or sterile normal saline solution.
(4) Treat the emulsion thus obtained in one of the following ways:
  (a) Dilute 0.5 cc. of the emulsion with 5.0 cc. of bouillon, and heat for 1 hour at 60°C. in each of the cases listed below.
  (b) Dilute as above, but heat at 70°C. for 1 hour.
  (c) Boil the emulsion for 5 minutes and then centrifuge to separate the clear fluid extract from the bacterial bodies.
  (d) Add 0.5 cc. of the emulsion which had been heated to 60°C. for 1 hour to two tubes of bouillon. Place in the ice box for one week. Centrifuge one tube and use the clear liquid and also the centrifuged suspension.
(5) Add from 0.1 to 0.5 cc. of one of the emulsions or extracts to a tube of bouillon.

Sterilization: Sterilization of bacterial extracts are given in step (4). Sterilization of bouillon not specified.

Use: To study nutrition of *Bacillus influenzae.* Author reported that growth appeared earlier and was heavier in bacterial extract bouillon than in blood bouillon. Using *Bacillus proteus* extract growth of *Bacillus influenzae* occurred only in 2 or 3 generations.

Reference: Thjötta (1921 p. 765).

944. Hitchens' Basal Sugar Free Agar Solution

Constituents:

1. Sugar free bouillon	1000.0 cc.
2. Agar (0.1%)	1.0 g.

Preparation:

(1) Dissolve 0.1% washed or purified agar in sugar free bouillon.
(2) Tube.
(3) Add 1.0% of the desired added nutrients. These may be added before sterilization or made up in concentrated solutions, sterilized and then added to sterile (2).

Sterilization: Method not given.

Use: Cultivation of anaerobes. The tubes are heated until a control tube containing 1.0 cc. of 1.0% methylene blue shows considerable reduction, then cooled to 45°C. until inoculated.

Added nutrients: 1.0% of any desired carbohydrate, alcohol, etc. may be used.

Variants: Andrade and phenol red may be used as indicators.

Reference: Hitchens (1922 p. 36).

945. Hegner and Becker's Blood Agar Solution

Constituents:

1. Water	270.0 cc.
2. NaCl (0.85%)	2.3 g.
3. Nutrient agar (2.0%)	30.0 cc.
4. Blood, rabbit	

Preparation:

(1) Add 30.0 cc. of ordinary 2.0% nutrient agar (pH 7.6) to 270.0 cc. of 0.85% saline solution (pH 7.6).
(2) Tube in 10.0 cc. quantities.
(3) Add 20 drops of blood directly from a rabbit's ear to each tube of sterile, melted and cooled tube of (2).
(4) Incubate 24 hours to test sterility.

Sterilization: Sterilize (2) at 120°C. in the autoclave.

Use: Cultivation of *Embodomonas intestinalis.* This medium is a modified Noguchi's serum medium described by Wenyon.

Reference: Hegner and Becker (1922 p. 17).

946. Thjötta and Avery's Blood Cell Bouillon

Constituents:

1. Bouillon.
2. Red blood cells.

Preparation:

(1) Prepare plain bouillon.
(2) Adjust the reaction of (1) to pH = 7.8.
(3) Wash the red cells from 20.0 cc. sterile defibrinated blood three times, each time with 50.0 cc. sterile salt solution. The cells are removed by centrifugation.
(4) Make up the cells in sterile distilled water to the original volume of blood.
(5) Dilute (4) with (2) in varying proportions from 1:10 to 1:100,000.

Sterilization: Sterilization of bouillon not given.

Use: To study bacterial nutrition of *Bacillus influenzae*. Author reported no growth in dilution of 1 to 10,000 of blood cells.

Reference: Thjötta and Avery (1921 p. 109).

947. Smith, Brown and Walker's Tissue Bouillon

Constituents:

1. Bouillon.
2. Tissue, Rabbit or Guinea Pig.

Preparation:

(1) Prepare ordinary bouillon.
(2) Tube in fermentation tubes.
(3) Kill a rabbit or guinea pig by chloroform.
(4) Moisten the animal thoroly with water.
(5) Reflect the skin and open the abdomen with sterile instruments.
(6) Remove the liver, spleen, kidneys and bits of tissue with sterile forceps.
(7) Place these bits of tissue, just large enough to pass into the narrow part of the fermentation tubes. The tissue is pushed into this narrow portion, but not necessarily beyond.
(8) Incubate two or more days to test sterility.

Sterilization: Sterilization of bouillon not given.

Use: Cultivation of anaerobes and flagellates.

Variants:

(a) Wrzosek reported that the addition of sterile tissue to a medium permitted the growth of obligate anaerobes.
(b) Harrass sterilized at 100°C. for 90 to 120 minutes the medium after the addition of animal tissue. He also suggested the use of water instead of bouillon.
(c) Gózony diluted equal parts of bouillon (containing 2.9% peptone) and water, and made slightly alkaline by the addition of sodium carbonate. After sterilization in the autoclave, cooled to 45°C. and a small piece of sterile dog kidney was added to each tube under aseptic conditions. The medium was used for the cultivation of flagellates.
(d) Bull and Pritchett added fragments of sterile skeletal muscle of the pigeon or rabbit to 100.0 cc. quantities of bouillon. They used the medium for the production of toxin by *Bacillus welchii*. Medium incubated in a vacuum jar from which the oxygen has been removed. They also used the above medium with the addition of 0.0 to 1.0% glucose, 0.2 or 0.3% giving the best results.

References: Smith, Brown and Walker (1905–06 p. 196), Wrzosek (1905 pp. 1268–1270), Harrass (1906 p. 2237), Bull and Pritchett (1917 pp. 129, 871, 873, 875), Gózony (1920 p. 566).

948. Orr's Glucose Heart Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Glucose (1.0%)	10.0 g.
3. Heart, beef	100.0 g.
4. Marble	

Preparation:

(1) Prepare 1.0% glucose bouillon.
(2) Add (1) to 100.0 g. of finely minced beef heart.
(3) Add a quantity of marble chips.
(4) Adjust to pH = 8.0.
(5) Cover with a layer of paraffin oil.

Sterilization: Method not given.

Use: Cultivation of *B. botulinus* and toxin production. Author reported that M.L.D. varied from 0.0001 cc. to 0.05 cc. depending on the strain used.

Reference: Orr (1920–21 p. 128).

949. Park, Williams and Krumwiede's Meat Medium

Constituents:

1. Bouillon.
2. Meat.

Preparation: (1) Drop pieces of meat into tubes of bouillon.

Sterilization: Method not given.

Use: General culture medium.

Reference: Park, Williams and Krumwiede (1924 p. 125).

950. Kligler's Tissue Infusion Bouillon

Constituents:

1. Bouillon.
2. Physiological salt solution.
3. Tissue.

Preparation:
(1) Prepare bouillon.
(2) Obtain tissue (beef heart, rabbit or cat tissue or organ or mucosa of various organs were used) as free from blood as possible under aseptic conditions.
(3) Wash (2) with saline solution to remove all visible traces of blood.
(4) Weigh and chop into small bits and suspend in 9 times the weight of saline solution.
(5) Shake thoroly and place in the ice box over night.
(6) Centrifuge and filter thru a Berkefeld candle.
(7) Tube (1) in 5.0 cc. lots.
(8) Add from 0.01 cc. to 1.0 cc. of sterile (6) to each tube of sterile (7).

Sterilization: Sterilize (6) by filtering thru a Berkefeld filter. Sterilization of (7) not specified.

Use: To show influence of growth accessory substances on growth of pathogenic bacteria.

Reference: Kligler (1919 p. 32).

951. Capaldi's Egg Yolk Bouillon

Constituents:
1. Bouillon.
2. Egg yolk.

Preparation:
(1) Break an egg. Separate the white from the yolk and place the yolk in a sterile Petri dish.
(2) Exact method of preparation of bouillon not given.
(3) Place a glowing glass rod on a spot on the covering of the egg yolk. (This sterilizes the covering of the naturally sterile yolk.)
(4) Remove 3–4 loops ("oesen"), (exact amount not given) of egg yolk thru the portion of covering of the yolk that had been sterilized and add to each tube of (3).

Sterilization: Sterilization of bouillon not given.

Use: Cultivation of tubercle bacilli and diphtheria bacilli to study toxin production by diphtheria bacilli.

Reference: Capaldi (1896 p. 801).

952. Oberstadt's Egg Albumin Bouillon

Constituents:
1. Bouillon.
2. Egg albumin.

Preparation:
(1) Boil eggs 10 minutes.
(2) Shell the egg, separate the yolk and cut the white into small pieces.
(3) To 100.0 cc. of clear ordinary nutrient bouillon that is slightly alkaline to litmus add the finely chopped albumin from one egg.
(4) Heat at 100°C. for 30 minutes and allow to stand until the following day.
(5) Filter off the egg albumin.
(6) Distribute the filtrate as desired.

Sterilization: Autoclave at 0.5 atmospheres pressure for 45 minutes. If the medium is not to be used within 8 days, sterilize the medium at 0.5 atmospheres pressure without filtering the egg albumin. Then filter just before ready to use.

Use: General culture medium. Author reported that growth of pathogenic and saphrophytic organisms was much better on this medium than on ordinary bouillon.

References: Oberstadt (1914–15 p. 137), Klimmer (1923 p. 202).

953. Lipschutz's Egg Albumin Bouillon

Constituents:

1. Water	100.0 cc.
2. Egg albumin	2.0 g.
3. Bouillon	200.0 to 300.0 cc.

Preparation:
(1) Prepare a 2.0% egg albumin solution in water.
(2) Add 20.0 cc. of 0.1 normal lye solution for each 100.0 cc. of (1).
(3) Allow to stand for 30 minutes shaking occasionally.
(4) Filter and distribute into Erlenmeyer flasks in 30 to 50.0 cc. lots.
(5) Mix 1 part sterile (4) with 2 or 3 parts sterile bouillon.

Sterilization: Sterilize (4) by placing on an asbestos plate and bringing to a boil. This may be done on the same or successive days, or the solution may be sterilized in the steamer. Method of sterilization of bouillon not given.

Use: Cultivation of gonococci.

Reference: Lipschütz (1904 p. 744).

954. Kahn's Casein Digest Egg Albumin Bouillon

Constituents:

1. Water	1000.0 cc.
2. Bouillon.	
3. Casein	200.0 g.

Preparation:

(1) Boil a few eggs for 15 minutes.
(2) Carefully separate the white from the yolk and wash in distilled water.
(3) Cut the washed coagulated albumin in cubes of about 2 mm.
(4) Place a cube into each of several test tubes.
(5) Prepare casein digest according to the method given by Kahn (see 648).
(6) Add 10.0 cc. of (5) to each tube.
(7) Seal the tube with a vaseline cap.

Sterilization: Autoclave at 15 pounds pressure for 20 minutes.

Use: To study proteolysis by spore forming anaerobes.

Variants:

(a) Torrey and Kahn added 1.0 cc. of casein digest containing 1.0% glucose or lactose to each tube, or they added 20.0% lactose, or whey broth was added to the casein digest bouillon. The authors reported that in a proper medium *Bacterium acidophilus* inhibited proteolysis by several spore bearing proteolytic anaerobes including *B. sporogenes*, *B. histolyticus* and *B. botulinus* for a period of 10 days or more.

Reference: Kahn (1922 p. 174), Torrey and Kahn (1923 p. 483).

955. Besredka and Jupille's Egg Bouillon (Besson)

Constituents:

1. Bouillon	500.0 cc.
2. Egg white (10.0% soln.)	400.0 cc.
3. Egg yolk (10.0% soln.)	100.0 cc.

Preparation: (1) Mix 5 parts of bouillon with 4 parts of a 10.0% egg white solution and 1 part of 10.0% egg yolk emulsion in sterile water.

Sterilization: If the egg white and yolk are not obtained under aseptic conditions, sterilize by heating at 55°C.

Use: Cultivation of pneumococci, gonococci, meningococci, various other highly parasitic forms as well as saprophytic organisms.

Variants:

(a) Stitt prepared the medium as follows:
(1) Prepare a 10.0% egg white solution by constantly heating the egg white while distilled water is added, little by little.
(2) Filter (1) thru absorbent cotton.
(3) Heat to 100°C.
(4) Filter thru filter paper.
(5) Tube or flask.
(6) Sterilize at 115° for 20 minutes.
(7) Add 1.0 cc. of normal NaOH, to 100.0 cc. of a 10.0% emulsion of egg yolk in water. Add sufficient NaOH to slightly clarify, but still be opaque when in rather thick layers.
(8) Heat to 100°C.
(9) Filter.
(10) Sterilize at 115°C. for 20 minutes.
(11) Mix 100.0 cc. sterile bouillon (containing no NaCl) with 80.0 cc. of (6) and 20.0 cc. of (10) under aseptic conditions.
(12) Distribute in sterile tubes or flasks.

Stitt reported that tubercle bacilli grew better if the peptone be omitted from the bouillon.

(b) Klimmer prepared the medium as follows:
(1) Thoroly mix 1 part egg white with 10 parts distilled water.
(2) Boil.
(3) Filter.
(4) Sterilize (method not given).
(5) Thoroly mix 1 part egg yolk with 10 parts distilled water.
(6) Add 1.0 cc. normal soda solution to 100.0 cc. (5).
(7) Boil.
(8) Filter.
(9) Sterilize (method not given).
(10) Mix 4 parts (4) with 1 part (9) and 5 parts nutrient bouillon.

References: Besson (1920 p. 55), Stitt (1923 p. 34), Klimmer (1923 p. 202).

956. Robertson's Alkaline Egg Bouillon

Constituents:

1. Water	1000.0 cc.
2. Eggs	4
3. NaOH (normal)	12.0 cc.
4. Bouillon	

Preparation:
(1) Mix the yolks of 2 eggs, the whites of 4 eggs and 12.0 cc. normal NaOH in 1000.0 cc. of water.
(2) Add 1 part (1) to 5 parts bouillon.

Sterilization: Method not specified.

Use: Cultivation of cholera organism.

Reference: Park, Williams and Krumwiede (1924 p. 126).

957. Piorkowski's Alkaline Egg Albumin Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Glucose (1.0%)	10.0 g.
3. Peptone (2.0%)	20.0 g.
4. Egg albumin (2.0% dry)	
5. NaOH	

Preparation:
(1) Prepare a 1.0% glucose bouillon containing 2.0% peptone.
(2) Distribute in 5.0 cc. lots.
(3) Prepare a 2.0% solution of dry egg albumin in tap water.
(4) Add 1.0 cc. (3) and 20.0% of a N/10 NaOH solution.

Sterilization: Method not given.

Use: Enrichment of streptococci from the blood.

Reference: Piorkowski (1922 p. 69).

958. Hibler's Blood Bouillon

Constituents:
1. Bouillon.
2. Blood, dog.

Preparation:
(1) Collect dog blood in sterile test tubes in about 5 cc. lots. (Blood may be collected in beaker and then the finely divided clot with a corresponding amount of serum, be distributed in tubes.
(2) Add a few drops of bouillon (or water).

Sterilization: Sterilize by heating at 58 or 97°C. Before use heat the tubes for 15 minutes in flowing steam.

Use: Cultivation of anaerobes and various parasitic forms. Author reported that the anaerobes grew better in a medium sterilized at 58°C. Fresh blood gave best results.

Variants:
(a) Davis mixed one-third volume of fresh rabbit blood with two thirds volume of bouillon to cultivate Ducrey's bacillus (chancroid bacillus).
(b) Wollstein cultivated Pfeiffer bacillus on a medium prepared as follows:
  (1) Boil fresh rabbit blood for 2 minutes in a water bath.
  (2) Centrifuge.
  (3) Add 0.5 cc. of the pale pink or yellow fluid resulting from (2) to 20.0 cc. of bouillon adjusted to pH = 7.8.
(c) Tanner and Besson added 0.5, 0.3 or 0.25 the volume of blood (or serum) to bouillon.
(d) Besson prepared a medium for the cultivation of pneumococci, meningococci and gonococci as follows:
  (1) Collect 400.0 cc. of beef blood in a sterile flask containing 30.0 cc. of a 1.0% ammonium citrate.
  (2) Shake thoroly.
  (3) Store for several hours at room temperature.
  (4) Dilute (3) with 3 volumes of sterile physiological salt solution.
  (5) Add 1 part (4) to 15 parts sterile bouillon.
(e) Thjötta and Avery boiled whole blood for 10 minutes, centrifuged and diluted the supernatant fluid 1 to 10, to 1:10,000 with bouillon adjusted to pH = 7.8. He reported no growth with dilutions of 1:1000 or greater.

References: Hibler (1899 p. 604), Davis (1903 p. 405), Wollstein (1919 p. 556), Tanner (1919 p. 47), Besson (1920 p. 31), Thjötta and Avery (1921 p. 109).

959. Dieudonné's Alkaline Blood Bouillon

Constituents:

1. Bouillon	500.0 cc.
2. Blood (defibrinated beef)	250.0 cc.
3. KOH (normal)	250.0 cc.

Preparation:
(1) Add 250.0 cc. of normal KOH to 250.0 cc. defibrinated beef blood.
(2) Adjust nutrient bouillon (or peptone solution) to neutral point to litmus.

Sterilization: Sterilization not specified. Author mentioned that (1) might be sterilized in the autoclave.

Use: Enrichment of cholera vibrio.

Variants: Kraus, Zia and Zubrzciky incubated the flasks for 3 hours at 50°C. for 24 hours at 37°C. before use.

References: Dieudonné (1909 p. 108), Kraus, Zia and Zubrzciky (1911 pp. 1084–1085).

960. Orcutt and Howe's Fat Blood Bouillon

Constituents:
1. Bouillon.
2. Blood, whole defibrinated.
3. Cream.

Preparation:
(1) Distribute sterile bouillon in 1.0 cc. lots in sterile test tubes.
(2) Add 1.0 cc. of defibrinated blood to each tube.
(3) Allow milk to stand in sterile test tubes.
(4) Remove the cream for (3).
(5) Dilute sterile (3) 1:10 with sterile bouillon.
(6) Add 0.02 cc. of (5) to each tube of (3).

Sterilization: Sterilization of bouillon not specified. Sterilize (4) by steaming at 100°C. for 20 minutes on each of 3 successive days.

Use: To study the influence of fat on hemolysis by staphylococci. Authors reported that with the organism studied hemolysis took place in the presence of whole milk, cream, butter, olive oil and triolein, but not in the presence of fat free milk, nut butter, triacetin, tributyrin or pork fat.

Variants: The authors used one of the following instead of cream:
(a) Remove the cream from milk by centrifugation. Add 0.01 cc. of the milk, after it has been sterilized by the fractional method in steam, to each tube of (3) above.
(b) Make a suspension of one of the fats (olive oil, butter, nut butter, triacetin, triolein, tributyrin and pork fat were used) by shaking 1.5 cc. of the fat in 10.0 cc. bouillon for one to 2 hours using a mechanical shaker. Add sufficient of one of these emulsions to bouillon so that each cubic cm. of bouillon shall contain 0.002 cc. of fat.

Reference: Orcutt and Howe (1922 p. 412).

961. Fildes' Pepsinized Blood Bouillon

Constituents:

1. Bouillon	
2. Saline solution	150.0 cc.
3. Blood, defibrinated, sheep	50.0 cc.

Preparation:
(1) Place 150.0 cc. of saline solution, not distilled water, 6.0 cc. pure HCl, 50.0 cc. defibrinated sheep blood and 1.0 g. pepsin (B.P. granulated) in a 250.0 cc. bottle fitted with a well ground glass stopper. Add ingredients in order specified.
(2) Shake thoroughly.
(3) Place in a water bath at 55°C. for 2 to 24 hours, shaking occasionally at first.
(4) Add 12.0 cc. of 20.0% NaOH.
(5) Adjust the reaction to pH 7.6 using cresol red as an indicator (permanganate color).
(6) Add pure HCl, drop by drop until pH 7.0 to 7.2 is reached, using phenol red as an indicator (red).
(7) Add 0.25% chloroform and dissolve by shaking.
(8) When ready for use, shake the flask and remove the pepsinized blood with a sterile pipette. Add from 2.0 to 5.0% to bouillon.

Sterilization: Not specified.

Use: Cultivation of *B. influenza.*

Reference: Fildes (1920 p. 129).

962. Dunham's Glucose Serum Bouillon

Constituents:

1. Water	300.0 cc.
2. Bouillon.	
3. Glucose	10.0 g.
4. Serum (sheep)	100.0 cc.
5. Litmus (1.0% solution of Kahlbaum)	

Preparation:
(1) Prepare bouillon.
(2) Dissolve 10.0 g. of glucose in (1).
(3) Dilute 1 part sheep serum with 3 times its volume of water.
(4) Mix 1 part of sterile (3) with 3 parts sterile (2).
(5) Add a sufficient quantity of sterile 1.0% Kahlbaum's purified litmus solution to impart a distinct color to the whole.

(6) Incubate 2 or 3 days to detect any accidental contamination.

Sterilization: Sterilize (2), (3) and litmus solution separately in the Arnold on each of 3 successive days.

Use: To study fermentation by diplococci.

Reference: Dunham (1906 p. 19).

963. Shmamine's Liver Serum Bouillon

Constituents:

1. Bouillon	100.0 cc.
2. Sodium nucleate	1.0 to 2.0 g.
3. Physiological salt soln.	20.0 cc.
4. Serum, horse	100.0 cc.
5. Liver, rabbit	

Preparation:

(1) Dissolve 1 to 2.0 g. of the sodium salt of nucleic acid in 20.0 cc. of physiological salt solution.
(2) Boil in the water bath for 15 minutes.
(3) Mix (2) with 100.0 cc. of sterile clear transparent horse serum.
(4) Add a small piece of rabbit liver that has been burned off in the flame.
(5) Mix equal parts sterile bouillon and (4).
(6) Following final sterilization pass CO_2 thru the tubes for 2 minutes and then seal with rubber stoppers and paraffin. (The fact that the medium was to be tubed and a small piece of rabbit liver added to each tube was not specified.)

Sterilization: Method of sterilization of serum or bouillon not specified. Sterilize (5) for one hour on each of 3 successive days at 60°C.

Use: Cultivation of spirochetes.

Reference: Shmamine (1912 p. 323).

964. Besson's Serum Bouillon

Constituents:

1. Bouillon.
2. Serum.

Preparation: (1) Mix one-half, one-third or one-fourth the amount of sterile serum with ordinary sterile bouillon under aseptic conditions.

Sterilization: Not specified.

Use: General culture medium.

Variants:

(a) Kligler and Robertson cultivated *Spirochaeta obermeieri* on a medium prepared as follows:
  (1) Mix one part rabbit serum with 2 parts physiological salt solution.
  (2) Prepare a bouillon containing 10.0% peptone.
  (3) Add 1.0% of (2) to (1).
  (4) Adjust (3) to pH = 7.2.
  (5) Distribute into 3 to 4.0 cc. lots into test tubes approximately 1.0 cm. in diameter.
  (6) Inoculate with one drop of infected blood, or if subcultures are being made, add a drop of fresh rabbit blood and rotate the tube gently. (This furnishes the necessary fibrin. Instead of fibrin, 0.05 to 0.1% agar may be used.)
  (7) Cover with a layer of oil about 1.5 cm. high.
  (8) Incubate at 28–30°C.
(b) Klimmer added one part serum to 3 to 20 parts bouillon, under aseptic conditions.
(c) Stitt cultivated *Borrelia recurrentis* on a medium prepared as follows:
  (1) Mix three parts of a 1.0% peptone bouillon with one part rabbit or horse serum (ascitic fluid may be used).
  (2) Tube in tall tubes.
  (3) Cover with a layer of oil not to exceed 1.5 cm. in height.

References: Besson (1920 p. 31), Kligler and Robertson (1922 p. 315), Klimmer (1923 p. 200), Stitt (1923 p. 54).

965. Veillon's Ascitic Fluid Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Ascitic fluid	1000.0 cc.

Preparation:

(1) Adjust the reaction of bouillon to be slightly alkaline.
(2) Add an equal amount of sterile ascitic fluid to sterile (1).

Sterilization: Method of sterilization of bouillon or ascitic fluid not given.

Use: Cultivation of gonococci and other highly pathogenic organisms.

Variants:

(a) The author mixed bouillon with $\frac{1}{3}$ its volume of ascitic fluid.
(b) Elser and Huntoon isolated meningococci from blood or spinal fluid in a mixture of 1000.0 cc. of ascitic fluid

and 2000.0 cc. of bouillon with a reaction of +0.6 to phenolphthalein.

(c) Besson mixed $\frac{1}{2}$, $\frac{1}{3}$ or $\frac{1}{4}$ the volume of sterile ascitic fluid with sterile bouillon.

(d) Kligler and Robertson cultivated *Spirochaeta obermeieri* in a medium prepared as follows:

(1) Prepare a 10.0% peptone broth (exact method not given).

(2) Add 1.0% of (1) to undiluted ascitic fluid.

(3) Adjust to pH = 7.2.

(4) Distribute in 3.0 to 4.0 cc. lots into test tubes approximately 1.0 cm. in diameter.

(5) Inoculate with 1 drop of infected blood, or if subcultures are being made, add a drop of fresh rabbit blood and rotate the tube gently. (This furnishes the necessary fibrin.)

(6) Cover with a layer of oil about 1.5 cm. high.

They reported that the spirochetes grew well at 37°C. but degeneration changes tended to set in early. After the culture was well started, growth proceeded well at room temperature.

References: Veillon (1898 p. 24), Elser and Huntoon (1909 p. 382), Besson (1920 p. 31), Kligler and Robertson (1922 p. 315).

966. Kahn's Casein Digest Ascitic Fluid Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Casein (2.0%)	20.0 cc.
3. Ascitic fluid	200.0 cc.
4. $CaCO_3$	

Preparation:

(1) Preparation of bouillon not given.

(2) Mix 1 part ascitic fluid and 5 parts (1) adjusted to +0.3.

(3) Add $CaCO_3$ (amount not given). (This is Lyall's broth.)

(4) Add 2.0% casein digest as prepared by Kahn (see 648).

(5) Tube.

(6) Seal the tubes with a vaseline cap by boiling the medium and vaseline.

Sterilization: Not specified.

Use: To determine hemolysis by spore forming anaerobes. The medium was inoculated thru the liquid vaseline and cooled quickly, incubated for 24 hours and 0.1, 0.2, 0.4 and 0.5 cc. of the culture transferred to 1.0 cc. of a 5.0% suspension of washed fresh sheep erythrocytes. Add sufficient physiological salt solution to each tube to bring the volume to 20.0 cc. and incubate at 37°C. for one hour.

Reference: Kahn (1922 p. 198).

967. Lyall's Carbonate Ascitic Fluid Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Ascitic fluid	200.0 cc.
3. $CaCO_3$	

Preparation:

(1) Preparation of bouillon not given.

(2) Mix one part ascitic fluid and 5 parts of (1).

(3) Add $CaCO_3$ (amount not given).

Sterilization: Not specified.

Use: To determine hemolysis by streptococci. Grow the organism in the medium for 18 hours. Add a definite amount of the 18 hour broth culture to 1.0 cc. of a 5.0% solution of washed red blood cells of a sheep. Incubate in a water bath for 1 hour at 37.5°C. Author reported that three degrees of hemolysis are possible (1) complete solution of erythrocytes, (2) change of undissolved cell from a bright red to a dark brown due to a transformation of oxyhemoglobin to methemoglobin, but cells are not dissolved, (3) no hemolysis or change in color of the cells.

Reference: Lyall (1914 p. 497).

968. Roddy's Bile Bouillon

Constituents:

1. Bouillon	1000.0 cc.
2. Ox bile	1000.0 cc.

Preparation: (1) Mix equal parts of ox bile and bouillon.

Sterilization: Sterilize in the steam sterilizer for 20 minutes on each of 3 successive days.

Use: Culture medium for parasitic and saprophytic organisms.

Reference: Roddy (1917 p. 42).

969. Mayer's Mucin Bouillon

Constituents:

1. Bouillon.
2. Mucin (Merck).

Preparation:
(1) Dissolve Merck's mucin in bouillon. (Water or nutrient agar may be used instead of bouillon.)
(2) Concentrate the solution by slow evaporation to obtain the desired strength of mucin.

Sterilization: Not specified.

Use: Cultivation of parasitic and saprophytic bacteria.

Reference: Mayer (1899 p. 818).

970. Schloffer's Urine Bouillon

Constituents:

1. Bouillon	2000.0 cc.
2. Urine	1000.0 cc.

Preparation:
(1) Prepare bouillon.
(2) Mix 2 parts sterile bouillon and 1 part sterile urine.
(3) Tube or flask.

Sterilization: Obtain urine under sterile conditions. The urine may be collected in sterile flasks and then heated to 70° to 80°C. to sterilize.

Use: Cultivation of diphtheria bacilli. Authors reported that growth a little more luxuriant than on ordinary bouillon.

Reference: Schloffer (1893 p. 659).

971. Kligler's Nasal Secretion Bouillon

Constituents:
1. Bouillon.
2. Nasal secretions.

Preparation:
(1) Prepare bouillon.
(2) Obtain nasal washings by using 50.0 cc. warm saline solution for each washing of apparently normal individuals.
(3) Collect the washings in sterile bottles.
(4) Test sterility and tube in 2.0 cc. lots in small test tubes.
(5) Mix 0.1 cc. of (4) with 5.0 cc. of (1).

Sterilization: Method of sterilization of bouillon not given. Sterilize (3) by filtering thru a Berkefeld filter.

Use: To show influence of nasal washing on growth of pathogenic bacteria. Author reported that nasal secretions of some individuals stimulated the growth of some organisms. Saliva treated in a similar manner did not.

Reference: Kligler (1919 p. 40).

972. Thjötta and Avery's Yeast Ascitic Fluid Bouillon

Constituents:

1. Distilled water	400.0 cc.
2. Brewer's yeast	100.0 g.
3. Bouillon	
4. Ascitic fluid	

Preparation:
(1) Exact composition of bouillon not given.
(2) Adjust the reaction to pH = 7.8.
(3) Emulsify 100.0 g. of brewer's yeast in 400.0 cc. distilled water with the reaction of pH = 4.6.
(4) Boil over a free flame for 10 minutes.
(5) Allow to sediment at room temperature.
(6) Pipette off the clear supernatant extract and test sterility.
(7) Store in ice chest until ready for use. Readjust the reaction of the extract from pH = 7.3 to 7.5 just before use.
(8) Dilute hemoglobin free ascitic fluid with (2) in varying proportions from 1:2 to 1:100.
(9) Add varying amounts of the yeast extract (amount not given) to (8).

Sterilization: Sterilization of bouillon not given. Yeast infusion is sterilized in the process of preparation in step (4).

Use: To study bacterial nutrition of *Bacillus influenzae*. Author reported no growth of *B. influenzae* without yeast extract. Growth in dilutions of ascitic fluid in 1:2 and 1:5 only.

Reference: Thjötta and Avery (1921 p. 109).

973. Thjötta and Avery's Yeast Serum Bouillon

Constituents:

1. Distilled water	400.0 cc.
2. Brewer's yeast	100.0 g.
3. Bouillon	
4. Serum	

Preparation:
(1) Exact method of preparation of bouillon not given.
(2) Adjust the reaction of (1) to pH = 7.8.
(3) Emulsify 100.0 g. of brewer's yeast in 400.0 cc. distilled water with the reaction of pH = 4.6.
(4) Boil over a free flame for 10 minutes.

(5) Allow to sediment at room temperature.
(6) Pipette off the clear supernatant extract and test sterility.
(7) Store in ice chest until ready for use. Readjust the reaction of the extract from pH = 7.3 to 7.5 just before use.
(8) Separate serum from sterile defibrinated blood by repeated centrifugation. Serum is to be free from blood pigment.
(9) Dilute (8) in (2) in varying proportions from 1:10 to 1:10,000.
(10) Add yeast extract (7) (amount not given) to (9).

Sterilization: Sterilization of bouillon not given. Yeast infusion sterilized by boiling in step (4).

Use: To study bacterial nutrition of *Bacillus influenzae.* Authors reported no growth of *B. influenzae* without yeast extract. No growth when serum diluted 1:10,000.

Reference: Thjötta and Avery (1921 p. 109).

974. Thjötta and Avery's Yeast Blood Bouillon

Constituents:

1. Distilled water.............. 400.0 cc.
2. Brewer's yeast.............. 100.0 g.
3. Bouillon
4. Blood

Preparation:

(1) Exact method of preparation of bouillon not given.
(2) Adjust the reaction of (1) to pH = 7.8.
(3) Emulsify 100.0 g. of brewer's yeast in 400.0 cc. of distilled water with the reaction of pH = 4.6.
(4) Boil over a free flame for 10 minutes.
(5) Allow to sediment at room temperature.
(6) Pipette off the clear supernatant extract and test sterility.
(7) Store in the ice chest until ready for use. Readjust the reaction of the extract from pH = 7.3 to 7.5 just before use.
(8) Boil whole blood 10 minutes.
(9) Centrifuge repeatedly until all the coagulated proteins have been thrown down.
(10) Dilute the supernatant fluid from (9) in varying proportions from 1:10 to 1:10,000 with bouillon.
(11) Add yeast extract (7) (amount not given).

Sterilization: Sterilization of bouillon not given. Sterilization of yeast infusion and blood is effected in steps (4) and (8) in the preparation.

Use: To study bacterial nutrition of *Bacillus influenzae.* Author reported nearly as good growth without the yeast extract as with it added. No growth after blood dilution of 1:1000.

Reference: Thjötta and Avery (1921 p. 109).

975. Thjötta and Avery's Yeast Blood Cell Bouillon

Constituents:

1. Distilled water.............. 420.0 cc.
2. Brewer's yeast.............. 100.0 g.
3. Bouillon
4. Red blood cells

Preparation:

(1) Exact method of preparation of bouillon not given.
(2) Adjust the reaction of (1) to pH = 7.8.
(3) Emulsify 100.0 g. of brewer's yeast in 400.0 cc. distilled water with the reaction of pH = 4.6.
(4) Boil over a free flame for 10 minutes.
(5) Allow to sediment at room temperature.
(6) Pipette off the clear supernatant extract and test sterility.
(7) Store in ice chest until ready for use. Readjust reaction of the extract from pH = 7.3 to 7.5 just before use.
(8) Wash the red blood cells from 20.0 cc. sterile defibrinated blood three times, each time with 50.0 cc. sterile salt solution. The cells are removed by centrifugation.
(9) Take up the cells in sterile distilled water to the original volume of blood.
(10) Dilute (9) with (2) in varying proportions from 1:10 to 1:100,000.
(11) Add yeast extract (7) (amount not given).

Sterilization: Sterilization of bouillon not given. Sterilization of yeast infusion effected by boiling in step (4).

Use: To study bacterial nutrition of *Bacillus influenzae.* Authors reported growth in a blood cell dilution of 1:100,000.

Reference: Thjötta and Avery (1921 p. 109).

976. Thjötta and Avery's Yeast Hemoglobin Bouillon

Constituents:

1. Distilled water	410.0 cc.
2. Brewer's yeast	100.0 g.
3. Bouillon	
4. Hemoglobin (crystalline)	1.0 g.

Preparation:

(1) Exact method of preparation of bouillon not given.
(2) Adjust the reaction of (1) to pH = 7.8.
(3) Emulsify 100.0 g. of brewer's yeast in 400.0 cc. distilled water with the reaction of pH = 4.6.
(4) Boil over a free flame for 10 minutes.
(5) Allow to sediment at room temperature.
(6) Pipette off the clear supernatant extract and test sterility.
(7) Store in the ice chest until ready for use. Readjust the reaction of the extract from pH = 7.3 to 7.5 just before use.
(8) Prepare crystalline hemoglobin by the method of Walker and Williamson. J. Biol. Chem., 41: 75, 1920.
(9) Prepare a 10.0% solution of crystalline hemoglobin in water by weight.
(10) Dilute sterile (9) with sterile bouillon in varying proportions from 1:10 to 1:200,000.
(11) Add yeast extract (7) amount not given.

Sterilization: Method of sterilization of bouillon not given. Sterilization of yeast infusion effected by boiling in step (4). Filter the hemoglobin solution thru a Berkefeld filter to sterilize.

Use: To study bacterial nutrition of *Bacillus influenzae.* Authors reported no growth of *B. influenzae* without yeast extract. Growth when hemoglobin solution diluted 1:200,000 (with yeast extract present).

Reference: Thjötta and Avery (1921 p. 109).

977. Savini and Savini-Castano's Bacteria Blood Bouillon

Constituents:

1. Bouillon.
2. Glycerol.
3. Blood.
4. *Staphylococcus aureus.*

Preparation:

(1) Place 5.0 cc. of glycerol in a small Erlenmeyer flask containing glass beads.
(2) Wash the growth from 2 or 3 cultures of *Staphylococcus aureus* (24 to 48 hours cultures at 37°C.) and place in sterile (1). (Bouillon cultures may be used.)
(3) Place in a paraffin oven at 58 to 60°C. over night until the bacteria are killed.
(4) Add 3.0 to 4.0 cc. of blood freshly drawn under aseptic conditions to (3).
(5) Shake until the blood is coagulated completely.
(6) Heat at 58 to 60°C. for about an hour. (When heating here and in step (3) place the cotton stopper in the tube or flask lightly to allow evaporation of water.)
(7) Add sufficient of (6) to sterile bouillon until the bouillon takes on a weak red color.

Sterilization: Method of sterilization of glycerol not specified. Method of sterilization of bouillon not given.

Use: Cultivation of influenza bacilli.

Variants: The author prepared a similar medium using a hemoglobin solution instead of blood. The powdered hemoglobin was sterilized at 100°C. in the hot air sterilizer after drying in the desiccator. The powdered hemoglobin was crushed in a sterile mortar with solution obtained in step (3) above. Filter thru sterile filter paper. This solution was used instead of blood as indicated in step (6) and (7).

Reference: Savini and Savini-Castano (1911 p. 494).

SUBGROUP I-C. SECTION 14

Liquid media or basal solutions containing a digest or autolysate other than a commercial digest.

A_1. Containing digests of plant origin only.
B_1. Yeast digest employed.
C_1. No salts added.
  Kammen's Yeast Digest (Klimmer). 978
  Ayers and Rupp's Yeast Digest Solution 979
  Robertson's Glucose Asparagin Solution 980
  Kister's Yeast Digest 981
C_2. Salts added.
  Kligler's Yeast Autolysate Solution. 982
  Jotten's Yeast Autolysate Solution.. 983
  Abt and Blanc's Yeast Autolysate Solution 984
  Robertson and Davis' Yeast Autolysate Solution 985
B_2. Plant digests other than yeast employed.
A_2.* Containing digests of animal origin only.
B_1. Digests prepared by acid hydrolysis.
C_1. Gelatin hydrolyzed.
  Davis and Ferry's Hydrolyzed Gelatin Solution 986
  Capaldi and Proskauer's Hydrolyzed Gelatin Solution 987
  Capaldi and Proskauer's Mannitol Hydrolyzed Gelatin Solution 988
C_2. Casein hydrolyzed.
D_1. Infusions or extracts not added.
  Robinson and Rettger's Hydrolyzed Casein Solution 989
  Cannon's Hydrolyzed Casein Solution 990
  Berthelot's Hydrolyzed Casein Solution 991
D_2. Infusions or extracts added.
  Mueller's Hydrolyzed Casein Heart Solution 992
  Robinson and Rettger's Extract Hydrolyzed Casein Solution 993
C_3. Other materials hydrolyzed.
  Gröer and Srnka's Hydrolyzed Placenta Solution 994
  Boyd's Hydrolyzed Meat Solution.. 995
  Harvey's Hydrolyzed Meat Solution 996
  Frouin and Ledebt's Hydrolyzed Serum Solution 997
B_2. Digest prepared by enzyme hydrolysis.
C_1. Peptic digest only, (no tryptic digest) added.
D_1. Digested by means of minced stomach.
E_1. Stomach only, digested.
  Martin's Stomach Digest Solution (Martin's peptone) 998
E_2. Liver digested.
  Martin's Liver Digest Solution 999
  Seallards and Bigelow's Liver Digest Blood Solution 1000
  Dubovsky and Meyer's Liver Digest Solution 1001
E_3. Spleen digested.
  Emery's Spleen Digest Solution 1002
E_4. Blood clot digested.
  Stickel and Meyer's Blood Clot Digest Solution 1003
  Bramigk's Peptic Digest Solution.. 1004
  Harvey's Blood Clot Digest Solution 1005
E_5. Digests of a variety of other materials used.
  Martin's Stomach Digest Infusion Broth 1006
  Besredka and Jupille's Egg Stomach Digest Solution 1007
  Stickel and Meyer's Meat Digest Solution 1008
D_2. Digested by pepsin.
  Frieber's Gelatin Digest Solution.. 1009
  Frieber's Fibrin Digest Solution....1110
  Bramigk's Peptic Tryptic Digest Solution 1111
  Jensen's Milk Digest Solution 1112
C_2. Tryptic digest only, (no peptic digest) added.
D_1. Blood clots or blood digest specified.
  Stickel and Meyer's Trypsinized Blood Clot Solution 1113
  Spray's Blood Clot Digest Solution. 1114
  Löffl's Trypsinized Blood Solution. 1115
D_2. Heart digest specified.
  Gordon et al. Trypsinized Heart Solution (Tanner) 1116
  Harvey's Trypsinized Heart Solution 1117
D_3. Serum digest specified.
  Distaso's Trypsinized Serum Solution 1118
D_4. Meat digest specified.
  Hottinger's Trypsinized Meat Solution (Klimmer) 1119
  Peckham's Trypsinized Beef Solution 1120
  Harvey's Trypsinized Meat and Kidney Solution 1121
  Celozzi's Placenta Digest Blood Solution 1122

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* See next page for A_3.

Douglas Trypsin Broth (Hartley).. 1123
D_5. Casein or milk digest specified.
Harvey's Basal Trypsinized Casein Solution........................ 1124
Teruuchi and Hida's Trypsinized Casein Solution.................. 1125
Zipfel's Trypsinized Casein Solution.............................. 1126
Bacto Tryptophane Broth (Dehydrated)......................... 1127
Bacto Peptonized Milk (Dehydrated)......................... 1128
Mueller's Trypsinized Casein Solution............................ 1129
Cole and Onslow's Trypsinized Casein Solution.................... 1130
Berman and Rettger's Trypsinized Casein Solution.................. 1131
Norris' Trypsinized Caseinogen Solution........................ 1132
Cannon's Trypsinized Casein Solution (Norton and Sawyer)........ 1133
D_6. Digest of a variety of materials specified.
Duval and Harris' Tryptic Digest Solution......................... 1134
Harvey's Tryptic Digest Solution.. 1135
Stickel and Meyer's Tryptic Digest Solution........................ 1136
C_3. Both peptic and tryptic digests added.
Stickel and Meyer's Digest Solution 1137
Frieber's Digest Extract Solution.. 1138
A_3. Containing digests of both plant and animal origin.
Davis and Ferry's Hydrolyzed Gliadin Solution..................... 1139
Robinson and Rettger's Hydrolyzed Edestin Casein Solution.......... 1140

978. Kammen's Yeast Digest (Klimmer)

Constituents:

1. Water....................	20,000.0 cc.
2. Yeast....................	10,000.0 g.

Preparation:

(1) Autoclave 10,000.0 g. yeast with 20 liters of water for 2 hours at 1.5 to 1 atmospheres pressure.
(2) Allow to settle and decant the liquid.
(3) Add pepsin and about 10 liters of 0.5% HCl solution to the thick yeast residue from (2).
(4) Incubate (3) for 5 days at 37°C.
(5) Allow to settle and decant the liquid or filter.
(6) Neutralize the liquid by the addition of NaOH.
(7) Heat at 50° until all the liquid has evaporated.
(8) Heat at 100 to 105°C. for one hour.
(9) Pound to a powder.

Sterilization: Material remains sterile in powdered form.

Use: Substitute for meat peptone.

Variants:

(a) Klimmer added the supernatant fluid of (2) to the liquid obtained in step (6) and prepared a powder as indicated above.
(b) Klimmer dissolved 10.0 g. of the powder obtained above, 8.0 g. NaCl and 0.1 g. KNO_3 in a liter of water. He specified that this medium could be used as ordinary bouillon or might be used in the preparation of special media.

Reference: Klimmer (1923 p. 170).

979. Ayers and Rupp's Yeast Digest Solution

Constituents:

1. Water......................	1000.0 cc.
2. Yeast......................	100.0 g.

Preparation:

(1) Add 100.0 g. dry fresh yeast to 1000.0 cc. distilled water with 1.0 g. pepsin. Add HCl to obtain pH = 4.4.
(2) Incubate at 40° for 24 hours.
(3) Steam for 30 minutes.
(4) Filter.
(5) Dilute to 1000.0 cc. with distilled water.
(6) Adjust reaction to pH = 7.5.

Sterilization: Method not given.

Use: General culture medium. Author reported that this medium gave very good growth of the delicately growing streptococci.

Reference: Ayers and Rupp (1920 p. 96).

980. Robertson's Glucose Asparagin Solution

Constituents:

1. Distilled water (sterile)....	1000.0 cc.
2. Asparagin (Merck).........	3.4 g.
3. $CaCl_2$......................	0.1 g.
4. Glucose....................	20.0 g.
5. $MgSO_4$....................	0.2 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1 by boiling 3 minutes. Make up the loss of water.
(2) Adjust to pH = 7.4.
(3) Tube in 5 cc. quantities.

Sterilization: Heat in the autoclave at 20 pounds pressure for 30 minutes.

Use: To study the relation of substances formed by *B. coli* to the growth of yeast. The author reported that this medium did not support the continued growth of yeast. The addition of aqueous extracts of autolized yeast and grated carrot to the medium in high dilution permitted the growth of yeast, but caused no increased growth of *B. coli*. The filtrate of the synthetic medium on which *B. coli* had been grown for 68 generations, permitted the continued growth of yeast.

Reference: Robertson (1924 p. 396).

981. Kister's Yeast Digest

Constituents:

1. Water.
2. Yeast.

Preparation:

(1) Dissolve the proteins and extractives from dry yeast by means of water.
(2) Evaporate or condense the extract a thick syrupy mass. This composes the yeast extract.
(3) To obtain a yeast peptone subject the yeast extract to super-heated steam for some time (exact time not specified).
(4) Change the hydrolysed mass into a light yellow powder. This gives you a yeast peptone—soluble in water.

Sterilization: Material required no further sterilization.

Use: In the preparation of yeast media, the yeast extract replaces the meat extract and the yeast peptone replaces the Witte or commercial peptone.

Variants: Kister reported that Yeast peptone may also be prepared by an acid hydrolysis under pressure or by a pepsin hydrolysis, in an incubator, of the protein of the yeast after the separation of the extractive materials. Exact method not given.

Reference: Kister (1921–22 p. 478).

982. Kligler's Yeast Autolysate Solution

Constituents:

1. Water	1000.0 cc.
2. Yeast	200.0
3. NaH_2PO_4	2.0 g.

Preparation:

(1) Suspend 200.0 g. of drained or centrifuged brewers yeast in a liter of water.
(2) Add 2.0 g. NaH_2PO_4.
(3) Add N/1 NaOH to obtain pH = 6.1.
(4) Add 5.0 cc. chloroform.
(3) Shake well—incubate at 37° for 2 days, shaking occasionally.
(6) After incubation, adjust to pH = 7.4 and heat in water bath or Arnold for 30 minutes.
(7) Filter thru paper.
(8) Tube.

Sterilization: Sterilize in the autoclave.

Use: Substitute for meat or meat infusion media for the cultivation of colon-typhoid group and others.

References: Kligler (1919 pp. 183–186), Harvey (1921–22 p. 120).

983. Jotten's Yeast Autolysate Solution

Constituents:

1. Water.	
2. Yeast (brewer's)	10,000.0 g.
3. NaCl	0.8 %
4. KNO_3	0.01%

Preparation:

(1) Wash 10,000.0 g. of brewers yeast twice with 20 liters of water. Use a large centrifuge if possible.
(2) Add N/1 NaOH or $(NH_4)_2CO_3$ until the whole mass has turned brown. Shake thoroly when adding NaOH or $(NH_4)_2CO_3$.
(3) Allow to stand for 30 to 60 minutes. This dissolves some of the impurities.
(4) Add fresh cold water to (2). This causes the yeast to separate in flakes. (Amount of water not given.)
(5) Allow the yeast to settle for several hours.
(6) Remove the water from the yeast.
(7) Add fresh water (amount not given).
(8) Distribute in 5 liter flasks, and place at 45° for 48 hours.
(9) Pour off the brown liquid.

(10) Add water to the sediment and centrifuge.

(11) Remove the supernatant liquid of (10) and add to the liquid obtained in (8). This finally leaves about 35 to 40 liters of extract.

(12) Boil for 90 minutes in the steamer and make slightly alkaline to litmus.

(13) Filter thru paper or cotton.

(14) Add 8.0 g. NaCl, 0.1 g. KNO_3 and 0.2 g. Na_2CO_3 per liter.

Sterilization: Sterilize in the steamer on each of 3 successive days.

Use: Meat extract substitute in the preparation of general culture media.

Variants:

(a) The author evaporated (1) to a quick mass, similar to meat extract, using a "Faust-Heim" evaporating oven.

(b) Klimmer prepared the medium as follows:

  (1) Wash 10,000.0 g. of bottom beer yeast with 20 liters of water, twice, using the centrifuge if possible.

  (2) Add about 1.5 cc. normal NaOH or $(NH_4)_2CO_3$ to the residue in thin stream, stirring constantly. The liquid becomes dark brown.

  (3) Add cold tap water after 30 to 60 minutes.

  (4) Allow to settle and decant the yeast water.

  (5) Stir the yeast mass with water.

  (6) Distribute in 5 liter flasks and incubate at 45°C. for two days.

  (7) Pour off the brown colored liquid.

  (8) Thoroly wash the residue with water, and centrifuge out the residue.

  (9) Mix the liquid from (7) and (8). Total volume 30 to 40 liters.

  (10) Boil for one hour in the steamer.

  (11) Filter thru cotton or paper.

  (12) Neutralize to litmus.

  (13) Add 0.8% NaCl, 0.01% KNO_3 and 0.02% Na_2CO_3 to (12).

  (14) Sterilize on each of three successive days.

  (15) The liquid from (9) may be evaporated to a thick brown paste, and using 15.0 g. per liter to prepare the medium.

  Klimmer reported that the medium might be used instead of ordinary nutrient bouillon and give very good results. It might also be used as a basis for the preparation of special media. For the enrichment of cholera allow the autolysis to continue for 3 days instead of 2 as indicated in step (6).

Reference: Jotten (1920 p. 359), Klimmer (1923 p. 171).

984. Abt and Blanc's Yeast Autolysate Solution

Constituents:

1. Water.
2. NaCl (0.9%).
3. Yeast (Brewers).

Preparation:

(1) Wash yeast in water and decant after settling. Repeat this several times.

(2) Dry the yeast.

(3) Prepare a physiological salt solution containing 9.0 g. NaCl per 1000.0 g. water.

(4) Add 8 to 10 parts of dry washed yeast to each 100.0 cc. of (3).

(5) Incubate at 48 to 50° for 24 to 36 hours.

(6) Add two volumes of water to (5).

(7) Heat to boiling.

(8) Filter.

(9) Add soda to neutralize to phenolphthalein, or make slightly alkaline.

(10) Heat for 15 minutes at 115°C.

(11) Filter.

(12) Evaporate 10.0 cc. of the filtrate to dryness to determine the dry material. Calculate the dry material in 100.0 cc.

(13) Dilute (11) so as to have 2.1 or even 0.5 parts of dry material per 100.0 cc.

(14) Distribute as desired.

Sterilization: Sterilize at 110° for 15 minutes.

Use: General culture medium for colon-typhoid group, parasitic and saprophytic forms and others.

Variants: Robertson and Davis prepared a similar medium as follows:

(1) Grow yeast on dextrose agar slants.

(2) Suspend in sterile distilled water, throw down in centrifuge and wash in sterile distilled water 3 times.

(3) Kill yeast by heating at 70°C. for 10 minutes. (The suspension contained about 500,000 per cc.)

(4) Allow to autolyze for one week at 37.5°C.
(5) Pass thru a Berkefeld filter.
(6) Adjustment of reaction not given.
(7) Add sterile (method of sterilization not given) physiological salt solution to (5). Amount of each not given.

They used the medium to study the influence of vitamines on bacterial growth.

Reference: Abt and Blanc (1921 p. 452), Robertson and Davis (1923 p. 154).

985. Robertson and Davis' Yeast Autolysate Solution

Constituents:

1. Sterile distilled water to...	1000.0 cc.
2. Asparagin (Merck).........	3.4 g.
3. $CaCl_2$......................	0.1 g.
4. Dextrose..................	20.0 g.
5. $MgSO_4$....................	0.2 g.
6. K_2HPO_4..................	1.0 g.
7. NaCl......................	5.0 g.
8. Yeast.	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1 by boiling 3 minutes.
(2) Restore original volume with sterile distilled water.
(3) Adjust reaction to pH = 7.4.
(4) Tube.
(5) Grow yeast on dextrose agar slants.
(6) Suspend in sterile distilled water, throw down in centrifuge and wash in sterile distilled water 3 times.
(7) Kill yeast by heating 70°C. for 10 minutes. (This suspension contained about 500,000 per cc.)
(8) Allow to autolyze for 1 week at 37.5°C.

Sterilization: Sterilize (4) ty autoclaving at 20 pounds pressure for 30 minutes. Filter (8) thru a Berkefeld to sterilize.

Use: To study influence of vitamins on bacterial growth. Authors reported a luxuriant growth of yeast.

Reference: Robertson and Davis (1923 p. 154).

986. Davis and Ferry's Hydrolyzed Gelatin Solution

Constituents:

1. Distilled water............	1000.0 cc.
2. Hydrolyzed gelatin (syrup).	20.0 g.
3. Tryptophane...............	0.4 g.
4. Tyrosine..................	1.5 g.
5. Cystin....................	0.4 g.
6. NaCl......................	5.0 g.
7. K_2HPO_4..................	3.0 g.
8. $MgSO_4$....................	0.5 g.

Preparation:

(1) Hydrolyze gelatin with 25.0% H_2SO_4 for 24 hours on sand bath. (Temperature not given.)
(2) Add $Ba(OH)_2$ until alkaline and filter.
(3) Neutralize exactly the $Ba(OH)_2$ with 10.0% H_2SO_4. Filter.
(4) Test filtrate for presence of free Ba and SO_4 ions.
(5) Concentrate to a thick syrup in a vacuum.
(6) Dilute to 2.0% solid material with distilled water
(7) To each liter of (6) add 3, 4, 5, 6, 7 and 8.
(8) Adjust reaction to pH 8.0–8.2 with N/10 NaOH. See med. 474.

Sterilization: Heat for 20 minutes at 115°C.

Use: Cultivation of *Bact. diphtheriae* for toxin production. Authors reported that this medium was superior to most synthetic amino acid media. Amino acids used were chemically pure.

Reference: Davis and Ferry (1919 p. 231).

987. Capaldi and Proskauer's Hydrolyzed Gelatin Solution

Constituents:

1. Water......................	1000.0 cc.
2. NaCl (0.02%)..............	0.2 g.
3. $MgSO_4$ (0.01%)............	0.1 g.
4. K_2HPO_4 (0.2%)...........	2.0 g.
5. Gelatin (2.0%)............	20.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Boil gelatin for 6 hours in 1.0% HCl, H_2SO_4 or HNO_3.
(3) Add 2.0% of (2) to (1) (or water).

Sterilization: Not specified.

Use: General culture medium. Author reported that typhoid bacteria showed no growth in this medium.

Reference: Capaldi and Proskauer (1896 p. 460).

988. Capaldi and Proskauer's Mannitol Hydrolyzed Gelatin Solution

Constituents:

1. Water......................	1000.0 cc.
2. Mannitol (0.1%)...........	1.0 g.
3. Gelatin (2.0%)............	20.0 g.

Preparation:
- (1) Dissolve 2 in 1.
- (2) Boil gelatin in 1.0% HCl, H_2SO_4, or HNO_3 for 6 hours.
- (3) Add 2.0% of (2) to (1).

Sterilization: Not specified.

Use: Acid production by colon-typhoid group.

Reference: Capaldi and Proskauer (1894 p. 464).

989. Robinson and Rettger's Hydrolyzed Casein Solution

Constituents:

1. Water	1000.0 cc.
2. Casein	50.0 g.

Preparation:
- (1) Boil 50.0 g. of casein with 10.0% HCl under a reflux condenser until the solution no longer responds to the Biuret test.
- (2) Evaporate on the water bath until nearly all the HCl is removed.
- (3) Neutralize the remaining acid with NaOH. This solution is of dark brown color and is known as "Casein C."
- (4) Dissolve 50.0 g. of (3) in 1000.0 cc. of water.
- (5) Heat on water bath for 20 minutes.
- (6) Adjust the reaction neutral to litmus.
- (7) Distribute in 20.0 cc. lots in 150.0 cc. Erlenmeyer flasks.
- (8) After sterilization add 0.1% sterile glucose to one half of the flasks.

Sterilization: Sterilize at 12 to 14 pounds pressure for 15 minutes.

Use: Cultivation of *B. diphtheriae* and toxin production. Authors reported that the cultures were nearly non toxic.

Variants: The authors prepared a similar medium as follows:
- (1) Heat 100.0 g. casein with 400.0 cc. concentrated HCl for a few hours on water bath under a reflux condenser.
- (2) If charring occurs, dilute with 150.0 cc. water and heat over free flame for 8 hours.
- (3) Filter off humus residue.
- (4) Filtrate is biuret free and is evaporated on water bath with frequent renewal of water.
- (5) Decolorize (method not given).
- (6) Neutralize with NaOH
- (7) Mix 100.0 cc. (6) with 1000.0 cc. water.
- (8) Method of sterilization not given. This medium is inferior to opsine as a general culture medium.

Reference: Robinson and Rettger (1917 p. 364), (1918 p. 219).

990. Cannon's Hydrolyzed Casein Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Asparagin	5.0 g.
3. Ammonium lactate	5.0 g.
4. KH_2PO_4	2.0 g.
5. $MgSO_4$	0.2 g.
6. Casein	10.0 g.

Preparation:
- (1) Hydrolyze 10.0 g. of casein with 200.0 cc. of 10.0% H_2SO_4.
- (2) Keep on a water bath for 24 hours (temperature not given).
- (3) Neutralize by the addition of a saturated barium hydroxide solution and filter.
- (4) Evaporate the solution until the amino acids crystallize.
- (5) Dissolve 2, 3, 4 and 5 in 1000.0 cc. of distilled water.
- (6) Add the crystals of (4) to one half of (5).

Sterilization: Method not given.

Use: Rapid detection of indol production.

Variant: Tanner employed all the amino acid crystals in step (6) and not only one-half.

References: Cannon (1916 p. 535), Tanner (1919 p. 63).

991. Berthelot's Hydrolyzed Casein Solution

Constituents:

1. Water	1000.0 cc.
2. Casein	15.0 g.

Preparation:
- (1) Hydrolyze casein to amino acids by acid (Method not given).
- (2) Dissolve 15.0 g. of the amino acids obtained from the hydrolysis in 1000.0 cc. water.
- (3) Exactly neutralize with soda (Indicator not specified).

Sterilization: Sterilize with ether (Method not given).

Use: Toxin production by *Proteus vulgaris*.

Reference: Berthelot (1914 p. 916).

992. Mueller's Hydrolyzed Casein Heart Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl (1.0%)	10.0 g.
3. $MgSO_4$ (0.04%)	0.4 g.
4. $CaCl_2$ (0.02%)	0.2 g.
5. K_2HPO_4 (0.2%)	2.0 g.
6. Glucose (0.2%)	2.0 g.
7. Phenol red (0.02% solution)	80.0 cc.
8. Heart, beef	1.0 lb.
9. Casein.	

Preparation:

(1) Prepare beef heart infusion by heating 1.0 pound of beef heart in 500.0 cc. of tap water to boiling. Strain and filter.

(2) Decolorize (1) by boiling for 25 minutes with 10.0% "Norit," a commercial grade of wood charcoal.

(3) Filter thru paper.

(4) Prepare casein hydrolysate by boiling commercial casein 18 hours with a mixture of 6 times its weight of water and 3 times its weight of concentrated H_2SO_4.

(5) Free from H_2SO_4 by the addition of $Ba(OH)_2$. Wash precipitate with water and concentrate the filtrate and washings.

(6) The quantity of casein hydrolysate used in the following preparations is equivalent to 0.5 g. of the original casein.

(7) Dissolve 2, 3, 4, 5, 6 and 7 in 1000.0 cc. of water.

(8) Prepare the following solutions:

(a)	Decolorized infusion (1)	25.0 cc.
	Glucose salt solution (7)	25.0 cc.
	Casein hydrolysate (6) 0 to	0.5 g.
(b)	Water	25.0 cc.
	Glucose salt solution (7)	25.0 cc.
	Casein hydrolysate	0.5 g.

(9) Adjust to pH = 7.8.

(10) Filter if necessary and tube.

Sterilization: Sterilize at 10 pounds pressure for 10 minutes.

Use: To study food requirements of streptococci and pneumococci. Author reported growth with casein hydrolysate present. No growth in other solutions.

Reference: Mueller (1922 p. 327).

993. Robinson and Rettger's Extract Hydrolyzed Casein Solution

Constituents:

1. Water	1000.0 cc.
2. Casein	50.0 g.
3. Meat extract (Liebig's) (0.5%)	5.0 g.

Preparation:

(1) Boil 50.0 g. of casein with 10.0% HCl under a reflex condenser until the solution no longer responds to the Biuret test.

(2) Evaporate on the water bath until nearly all the HCl is removed.

(3) Neutralize the remaining acid with NaOH. This solution is of dark brown color and is known as "Casein C."

(4) Dissolve 50.0 g. of (3) in 1000.0 cc. water.

(5) Decolorize by filtering thru animal charcoal.

(6) Add 0.5% Liebig's meat extract.

(7) Heat on a boiling water bath for 20 minutes.

(8) Adjust the reaction to +0.6 to phenolphthalein, and filter.

(9) Distribute in 20.0 cc. lots in 150.0 cc. Erlenmeyer flasks.

Sterilization: Sterilize at 12 to 14 pounds pressure for 15 minutes.

Use: Cultivation of *B. diphtheriae* and toxin production.

Reference: Robinson and Rettger (1917 p. 363).

994. Gröer and Srnka's Hydrolyzed Placenta Solution

Constituents:

1. Water	4000.0 cc.
2. Placenta	4000.0 g.

Preparation:

(1) Mix about 4 liters (3 placenta to 1 liter on an average) placenta with the blood, cut into medium sized pieces, with 4 liters of water.

(2) Add about 10.0 cc. concentrated HCl and mix well.

(3) Boil for about 2 hours to a brown mixture, adding water.

(4) Strain and filter thru paper while still warm.

(5) Add 10.0% NaOH until phenol-

phthalein is turned red (hot titration) to the clear light greenish yellow filtrate.
(6) Boil for one hour.
(7) Filter off the albumin.
(8) Readjust the reaction (pH = 7.8).
(9) Distribute the crystal clear fluid into flasks.

Sterilization: Method not given.

Use: Cultivation of diphtheria bacillus and toxin production.

Reference: Gröer and Srnka (1918–19 p. 334).

995. Boyd's Hydrolyzed Meat Solution

Constituents:

1. Water, tap	1000.0 cc.
2. H_2SO_4 (strong)	100.0 cc.
3. NaCl	5.0 g.
4. KCl	2.0 g.
5. Lean meat	1.0 lb.

Preparation:
(1) To 300.0 cc. of tap water add 100.0 cc. strong H_2SO_4 gradually with constant stirring.
(2) Cut up 1 pound lean meat into small pieces and add to (1) after the reaction has subsided somewhat.
(3) Place (2) in a suitable water bath and bring quickly to a boil. Boil for 3 to 4 hours with frequent stirring.
(4) After 3 hours withdraw about 10.0 cc. and test for absence of proteoses with nitric acid. If negative withdraw another 5 or 10.0 cc. and carry out Biuret reaction. Whether hydrolysis is to be continued or not depends upon the color obtained.
(5) When the color is satisfactory withdraw the flask from the boiling water and add calcium hydrate (quicklime, CaO, that has been exposed to the free air for some time is best) until a large precipitate of $CaCO_4$ is formed. Continue adding lime until the precipitate occupies about $\frac{1}{3}$ of the fluid in the flask.
(6) Filter thru lint and wash thru the lint with a little tap water. Continue the neutralization of the filtrate obtained with more lime, best carried out in 3 stages so as to avoid an excess of precipitate at any one time.
(7) Neutralize the acid completely and make the medium faintly alkaline to phenolphthalein.
(8) Boil (7) for 15 minutes.
(9) Filter hot thru filter paper.
(10) Dilute to 1000.0 cc. by the addition of water.
(11) Dissolve 3 and 4 in (10).
(12) Make the desired final adjustment of the reaction.

Sterilization: Sterilize in the autoclave.

Use: General culture medium.

Variants:
(a) The author suggested the sterilization of solution (9) above by autoclaving and storing until ready for use. When desired continue as in steps (10), (11) and (12) as indicated.
(b) Norris prepared the medium as follows:
  (1) Add 500.0 g. lean meat to 40.0 cc. of strong H_2SO_4.
  (2) Pound together in a mortar.
  (3) Mix thoroly and digest in a water bath for 4 days at 70°C.
  (4) Test for proteose with nitric acid and then the Biuret test.
  The remainder of the preparation is identical with Boyd's medium, step (5) thru (12).

References: Boyd (1917–18 p. 410), Norris (1918–19 p. 176).

996. Harvey's Hydrolyzed Meat Solution

Constituents:
1. Water.
2. Meat, goat, sheep or ox.

Preparation:
(1) Mince finely fat-free goat, sheep or ox flesh.
(2) Add to it 80.0 cc. strong commercial hydrochloric acid of specific gravity 1.16, per kilogramme.
(3) Mix thoroly.
(4) Keep in water bath 4 days at 70°C.
(5) Bring the volume up to 1000.0 cc. for each kilogramme of minced meat used.
(6) Mix 1 part (5) with 2 parts boiling water.
(7) Add 75.0 cc. 40.0% sodium hydroxide for each kilogramme of minced meat used.
(8) Make the reaction faintly alkaline to litmus.

(9) Steam 60 minutes.
(10) Pour the mixture on to a wet, thick, clean cloth.
(11) Collect the fluid which drains thru the cloth together with that obtained by squeezing the cloth.
(12) Estimate and adjust the reaction of the medium to a definite pH value or to faintly alkaline to litmus or 1.0% acid to phenolphthalein.
(13) Filter thru well-wetted, thick, filter paper.
(14) Add 1 part of the filtrate to 2 parts boiling water.

Sterilization: Sterilize in the autoclave.

Use: General culture medium.

Reference: Harvey (1921–22 p. 69).

997. Frouin and Ledebt's Hydrolyzed Serum Solution

Constituents:

1. Water	1000.0	cc.
2. NaCl	6.0	g.
3. KCl	0.3	g.
4. Potassium phosphate	0.5	g.
5. $MgSO_4$	0.3	g.
6. $CaCl_2$	0.15	g.
7. Serum albuminoids	10.0	g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Hydrolyze 10.0 g. of serum albuminoids in (1). (Method not given.)
(3) Neutralize (indicator not specified).

Sterilization: Method not given.

Use: Cultivation of colon typhoid group.

Variants: The authors added 2.0% glucose or 3 to 4.0% glycerol to the medium.

Reference: Frouin and Ledebt (1911 p. 24).

998. Martin's Stomach Digest Solution (Martin's Peptone)

Constituents:

1. Water	1000.0	cc.
2. Stomach (hog)	200.0	g.

Preparation:

(1) Chop the stomachs of five hogs into small pieces.
(2) Mix 200.0 g. of (1), 10.0 g. pure HCl and 1000.0 cc. water at 50°C.
(3) Allow to stand at 50°C. for 12 to 24 hours.
(4) Heat at 100°C. (Time not specified.)
(5) Strain or filter thru a layer of absorbent cotton.
(6) Heat the filtrate and make alkaline when the temperature reaches about 80°C.
(7) Filter the fluid thru paper to clarify.
(8) Heat at 120°C.
(9) Filter.
(10) Distribute in flasks.

Sterilization: Sterilize by heating at 115° for 15 minutes.

Use: Cultivation of diphtheria bacilli and toxin production. The author reported that the addition of 2.0 g. acetic acid per liter favored toxin production. Other organisms grew well on this medium.

Variants:

(a) Nicolle, Debains and Jouan cultivated meningococci on a medium prepared as follows:
    (1) Heat one liter of water to about 55°C.
    (2) Add 10.0 cc. of pure HCl (22° Baum's) to (1).
    (3) Add 300.0 g. of finely divided hog's stomach.
    (4) Regulate the temperature to about 50°C. and keep at this temperature for 7 to 8 hours.
    (5) Heat to 80 or 90 degrees to destroy the pepsin and stop digestion.
    (6) The peptone thus obtained may be preserved for several weeks.
    (7) To use the peptone, make the peptone slightly alkaline to litmus and heat at 120°C.
    (8) Filter on a wet filter paper.
    (9) Add 2.0 g. of glucose.
    (10) Sterilize at 112 to 115°C.
(b) Besson used 250.0 g. hog's stomach instead of 200.0 g. and digested for 20 to 24 hours instead of 12 to 24 hours.
(c) Harvey digested for 20 hours and steamed for 30 minutes instead of heating at 120°C. as indicated in step (8) above.
(d) See medium 877, step (1) thru (8) for Park, Williams and Krumwiede's method of preparation.

References: Martin (1898 p. 32), Thoinot and Masselin (1902 p. 22), Nicolle, Debains and Jouan (1918 p. 151), Besson (1920 p. 28), Harvey (1921–22 p. 98), Park, Williams and Krumwiede (1924 p. 118).

999. Martin's Liver Digest Solution

Constituents:

1. Water	2000.0 cc.
2. Stomach (hog)	200.0 g.
3. Liver, beef or hog	200.0 g.

Preparation:

(1) Mix 200.0 g. of chopped hog stomach and 200.0 g. chopped hog or beef liver, and 20.0 cc. of commercial HCl in 2 liters of water.
(2) Digest (1) at 50°C. for 12 to 24 hours.
(3) Boil the liquid, at the end of 24 hours.
(4) Allow to cool and settle for 24 hours or less.
(5) Decant or siphon off the clear liquid.
(6) Neutralize and make slightly alkaline by the addition of soda. Indicator not specified.
(7) Heat at 120 degrees.
(8) Filter and distribute as desired.

Sterilization: Method not given.

Use: Cultivation of typhoid bacilli. Author reported that typhoid and para typhoid bacilli developed rapidly in this medium, producing gas, and acid and finally the reaction became alkaline.

References: Martin (1915 p. 261), Harvey (1921–22 p. 99).

1000. Sellards and Bigelow's Liver Digest Blood Solution

Constituents:

1. Water	1000.0 cc.
2. Veal	100.0 g.
3. Stomach (hog)	150.0 g.
4. Liver	200.0 g.
5. Blood, horse or rabbit (10.0%)	100.0 cc.

Preparation:

(1) Prepare a peptone by digesting 100.0 g. veal, 150.0 g. hog stomach and 200.0 g. liver in a liter of water containing 1.0% concentrated HCl, for 19 hours at 53°C.
(2) Heat in an Arnold to destroy the pepsin.
(3) Decant the supernatant fluid and neutralize at room temperature first to litmus and then to china blue and rosalic acid.
(4) Again decant and dilute with an equal volume of water.
(5) Add 10.0% horse or rabbit blood to sterile (4).

Sterilization: Sterilize (4) in the Arnold. Method of sterilization of blood not given. Sterilize the mixture of blood and digest for 15 to 20 minutes in the Arnold.

Use: Cultivation of measles virus from patients' blood.

Reference: Sellards and Bigelow (1920–21 p. 242).

1001. Dubovsky and Meyer's Liver Digest Solution

Constituents:

1. Water, tap	5000.0 cc.
2. Stomach, hog	400.0 g.
3. Liver	400.0 g.
4. K_2HPO_4 (0.2%).	
5. Heart	1000.0 g.

Preparation:

(1) Wash clean and mince finely 5 or more large hogs' stomachs.
(2) Mince an equal amount of clean hog or beef liver.
(3) Mix 400.0 g. (1), 400.0 g. (2), 40.0 g. HCl and 4000.0 cc. tap water at 50°C.
(4) Keep in glass or porcelain receptacles for 18–24 hours.
(5) Make Biuret and tryptophan test, when both are + the digest is green-yellowish and contains little undigested debris.
(6) Transfer to large bottles and steam 10 minutes at 100.0°C. to stop digestion.
(7) Strain the digest thru cotton or preferably store over night in the ice chest and decant after 24 hours.
(8) Warm the digest to 70°C. and neutralize with 2 N Na_2CO_3 using litmus.
(9) Filter the desired amount, add 0.2% K_2HPO_4 and adjust to pH = 7.4.
(10) Slowly heat to boiling finely ground 1000.0 g. fat-free heart and tap water 1000.0 cc.
(11) Adjust to pH = 8.0 or 8.2.
(12) Cool and carefully skim off the layer of fat which floats on medium.
(13) To each liter of (12) add 2 liters of (9).
(14) Adjust to pH = 7.2 or 7.4.

Sterilization: Sterilize at 18 pounds pressure for one hour. Incubate 5 days and repeat the same sterilization for one hour at 18 pounds pressure.

Use: Cultivation and toxin production by *B. botulinus*. Authors reported toxin production very good in this medium.

Reference: Dubovsky and Meyer (1922 p. 505).

1002. Emery's Spleen Digest Solution

Constituents:

1. Water	1500.0 cc.
2. Spleen (beef)	1500.0 g.
3. Stomach (hog)	250.0 g.

Preparation:

(1) Digest 250.0 g. of beef spleen pulp, 250.0 g. hog stomach with 1.5 liters of water acidulated with 15.0 cc. HCl for 24 hours.

(2) Add beef spleen pulp (500.0 g. to 1.0 liter of water) to (1).

(3) Boil 10 minutes.

(4) Filter thru wet absorbent cotton.

Sterilization: Not specified.

Use: Differentiation of coli and typhoid bacilli.

Reference: Emery (1901 p. 979).

1003. Stickel and Meyer's Blood Clot Digest Solution

Constituents:

1. Tap water	1000.0 cc.
2. Blood clots	100.0 g.
3. Pig's stomach (minced)	100.0 g.
4. K_2HPO_4	2.0 g.

Preparation:

(1) Obtain 10 liters fresh beef blood from the abattoir.

(2) Decant and store the serum (which has separated on standing) in a refrigerator.

(3) Weigh the blood clots and mix 100.0 g. with 1 liter tap water.

(4) Place mixture in an enameled pot, bring slowly to a boil and boil slowly for 5 minutes, stirring constantly.

(5) Wash and mince pig's stomach.

(6) Cool (4) to 50°C. and add 100.0 g. of minced pig's stomach for each liter of (4).

(7) Transfer to glass or porcelain receptable and finally add 1.0% HCl.

(8) Digest at 50°C. for 18–24 hours.

(9) Make a Biuret and tryptophane, test; when both are +, the digest is yellowish green and contains very little indigested debris.

(10) Transfer to large bottles and steam for 10 minutes to stop digestion.

(11) Strain thru cotton or preferably store over night in ice chest and decant after 24 hours.

(12) Warm (5) to 70°C. and neutralize with 2 normal Na_2CO_3 to litmus.

(13) Filter into a flask.

(14) Add the 2.0 g. K_2HPO_4.

(15) Adjust to desired reaction using litmus or preferably to a definite H-ion concentration (pH = 7.0 to 7.5).

(16) Clear (15) by adding 5–10.0% of the decanted beef serum. Steam 45–60 minutes.

(17) Remove (16) from steamer and allow the clot to form as a compact mass. Decant, or better centrifuge, the medium to remove it.

Sterilization: Sterilize at 100° for 30 minutes on 2 successive days.

Use: General inexpensive culture medium.

Reference: Stickel and Meyer (1918 p. 81).

1004. Bramigk's Peptic Digest Solution

Constituents:

1. Water	4000.0 cc.
2. Blood clot	1000.0 g.
3. Stomach (hog)	2

Preparation:

(1) Obtain a bucket of blood clots from the slaughter house.

(2) Wash the clots free from blood with running water.

(3) Press the clots free from water.

(4) To 1000.0 g. clots (contains about 230.0 g. fibrin) add 3 liters of water and 15.0 cc. of H_2SO_4.

(5) Allow to stand over night.

(6) Filter thru a sieve and press the water from the clot or fibrin.

(7) Add the clot to 3 liters water and 18.0 cc. H_2SO_4 heated to about 50°C.

(8) Remove the mucous membrane from two fresh pigs' stomachs.

(9) Run the stomachs thru a meat chopping machine.

(10) Mix (9) with 1 liter of water and 8.0 cc. H_2SO_4.

(11) Heat to 35°C.

(12) Mix (11) and (7) thoroly and place at 37°C. for 48 hours. Stir frequently. Some fat will settle out.

(13) Filter and neutralize with ammonia.
(14) Distribute in flasks and sterilize.

Sterilization: Method not given.

Use: General culture medium and substitute for commercial peptone.

Variants: The author prepared a dry material by treating the medium obtained above as follows:

(1) Neutralize the digest with $Ba(OH)_2$ (about 40.0 g.) and boil until the upper fluid is clear.
(2) The reaction should be slightly acid, showing a light excess of H_2SO_4.
(3) Allow the digest to settle.
(4) Pour off the clear solution and filter the remainder, washing the precipitate with water.
(5) This gives a light yellow solution containing about 10.0% peptone.
(6) This fluid may be evaporated over a water bath or in a vacuum to a thick syrupy paste and then drawn into threads and dried in a desiccator.
(7) This peptone is equal to Witte's peptone in the preparation of media.

Reference: Bramigk (1921 p. 429).

1005. Harvey's Blood Clot Digest Solution

Constituents:

1. Water	1000.0 cc.
2. Blood.	
3. Pig's stomach	100.0 g.
4. HCl	10.0 g.

Preparation:

(1) Procure ox or sheep blood from the slaughter house.
(2) Allow to clot.
(3) Decant and store the serum in an ice chest.
(4) Mince the clot.
(5) Add 100.0 g. clot to 1000.0 cc. tap water.
(6) Raise slowly to boiling temperature.
(7) Boil 10 minutes.
(8) Cool to 50°C.
(9) Add 100.0 g. minced pig's stomach and 10.0 cc. strong hydrochloric acid.
(10) Digest 20 hours.
(11) Make a biuret and a tryptophane test.
(12) Boil 10 minutes to stop the digestion.
(13) Pour the mixture on to a wet, thick, clean cloth.
(14) Collect the fluid which drains thru the cloth, together with that obtained by squeezing the cloth.
(15) Filter the fluid collected thru well-wetted, thick, filter paper.
(16) Bring the volume up to 1000.0 cc.
(17) Filter thru well wetted, thick filter paper.
(18) Make the reaction neutral to litmus.
(19) Add 75.0 cc. of the decanted serum (3).
(20) Steam sixty minutes.
(21) Allow the clot to form a compact mass.
(22) Decant the clear fluid.

Sterilization: Sterilize at 100°C.

Use: General culture medium.

Reference: Harvey (1921–22 p. 100).

1006. Martin's Stomach Digest Infusion Broth

Constituents:

1. Water	2000.0 cc.
2. Veal	500.0 g.
3. NaCl	5.0 g.
4. Stomach (hog)	200.0 g.

Preparation:

(1) Infuse 500.0 g. of finely ground veal with 1000.0 g. water for 20 hours at 35°C.
(2) Strain out the meat and add 5.0 g. NaCl to the fluid.
(3) Chop the stomachs of 5 hogs into small pieces.
(4) Mix 200.0 g. of (3), 10.0 g. HCl (pure) and 1000.0 cc. water at 50°C.
(5) Allow to stand at 50°C. for 12 to 24 hours.
(6) Heat at 100°C. (Time not specified).
(7) Strain or filter thru a layer of absorbent cotton.
(8) Heat the filtrate and make alkaline when the temperature reaches about 80°C.
(9) Filter the fluid thru paper to clarify.
(10) Mix 1 liter of (9) with 1 liter of (2).
(11) Heat to 70°C.
(12) Filter thru paper.
(13) Make alkaline (method not given).

Sterilization: Method not given.

Use: Cultivation of diphtheria bacilli and toxin production. Author reported that heating the medium decreased the ability of the medium to produce toxin. Heating only to 70°C. and filtering thru a Chamberland filter gave good results. Other investigators utilized a similar medium for the production of tetanus toxin.

Variants:

(a) Thoinot and Masselin used the stomachs of 5 hogs, but did not specify the use of 200.0 g. They also used 500.0 g. beef instead of veal.

(b) Bezançon digested the stomach for 24 hours instead of 12 to 24, infused beef instead of veal, heated the mixture at 115° for 15 minutes instead of heating at 70°C. in step (11) above and sterilized in the autoclave at 115° for 15 minutes.

(c) Besson used 250.0 g. hogs stomach instead of 200.0 g. and added 7.0 cc. of normal soda solution to the medium after it had been neutralized to litmus.

(d) Wilcox studied the production of tetanus toxin using one of the following combinations, prepared according to Martin's process:

(1) (a) 200 g. minced stomach in 1000.0 cc. water.
  (b) 400 g. minced stomach in 1000.0 cc. water.
  (c) 200 g. minced stomach in 1500.0 cc. water.
  (d) 300 g. minced stomach in 1000.0 cc. water.

(2) Veal infusions prepared from one of the following:
  (a) 500 g. veal to 1000.0 cc. water.
  (b) 500 g. veal to 500.0 cc. water.

(3) Add 1.0% glucose if desired.

Author reported that 1 part (1) (a) to 1 part (2) (a) gave highest toxin production with organism studied.

(e) Dopter and Sacquépée used beef instead of veal, added 7.0 cc. of normal soda to the medium after it had been neutralized to litmus and autoclaved at 117°C. for 15 minutes instead of heating at 70°C. as in step (11) above.

(f) Harvey neutralized the medium to litmus added 7.0 cc. of normal NaOH per liter, then filtered thru well-wetted thick filter paper, steamed 30 minutes, filtered again thru well-wetted thick filter paper while hot, distributed and sterilized.

(g) Klimmer used the stomachs of 5 hogs, but did not specify the use of 200.0 g. He carried on the digestion for 24 hours and then sterilized. Equal volumes of the sterile digest (1000.0 cc.) and the infusion from 500.0 g. of veal to 1000.0 cc. of water were mixed, boiled and neutralized. The reaction was made slightly alkaline, or adjusted to any desired pH value, filtered, tubed and sterilized in the usual manner.

(h) Park, Williams and Krumwiede infused the veal and 1000.0 cc. of water for 18 to 24 hours at 35°C., heated at 45 to 48°C. for one hour, then boiled briskly for 30 minutes and strained thru cheese cloth. This infusion at 70°C. was mixed with an equal volume of stomach digest at 70°C., prepared according to Martin's method, and heated to boiling. The reaction was adjusted to +0.5 to phenolphthalein at room temperature. The medium was sterilized at 15 pounds pressure for 30 minutes after the reaction was readjusted and filtered thru cotton and paper. They prepared a similar medium with a reaction of +1.0 to phenolphthalein and added 1.0% glucose. This medium was used for the production of tetanus toxin.

References: Martin (1898 p. 35), Thoinot and Masselin (1902 p. 23), Bezançon (1920 p. 111), Besson (1920 p. 29), Wilcox (1921 p. 414), Dopter and Sacquépée (1921 p. 119), Harvey (1921–22 p. 99), Klimmer (1923 p. 200), Park, Williams and Krumwiede (1924 p. 133).

1007. Besredka and Jupille's Egg Stomach Digest Solution

Constituents:

1. Distilled water.
2. Beef or veal......... 500.0 to 750.0 g.
3. Egg.
4. Stomach (hog).

Preparation:

(1) Beat the white of eggs with 10 volumes of distilled water, adding the water little by little.

(2) To clarify run thru a sieve covered with a thin layer of absorbent cotton.

(3) Heat to 100°C. and then filter on a Chardin paper.
(4) Distribute the opalescent fluid in flasks or tubes in 20.0 cc. lots.
(5) Dilute egg yolks with 10 volumes of distilled water.
(6) Clarify the egg yolk by the addition of normal soda solution. Generally 1.0 cc. of normal soda solution to 100.0 cc. suspension of egg yolk will give the proper solution. However, different yolks may require only half as much. The suspension should be slightly yellow in a thin layer.
(7) Heat at 100°C.
(8) Filter on Chardin paper.
(9) Distribute in flasks or tubes in 20.0 cc. lots.
(10) Prepare a bouillon from 500 to 750.0 g. chopped beef or veal with 1000.0 cc. water and Martin's peptone, by heating to boiling under a low flame and then boiling for 30 minutes. See med. (1006).
(11) Filter and make slightly alkaline.
(12) Heat at 120°C. to activate precipitation.
(13) Filter thru double paper.
(14) Distribute in flasks or Roux tubes.
(15) Add to 500.0 cc. of (14), 400.0 cc. of (4) (sterile egg white solution), and 100.0 cc. (9) (sterile egg yolk solution) under aseptic conditions.
(16) Distribute in sterile tubes in 10.0 cc. lots under aseptic conditions.

Sterilization: Sterilize (4) and (9) at 115°C. for 20 minutes. Sterilize (14) at 115°C. for 25 minutes.

Use: General culture medium, for highly parasitic and saprophytic forms. To cultivate tubercle bacilli, add cold, and under aseptic conditions, 20.0 cc. of (4) (egg white solution) and 5 to 20.0 cc. of (9) (egg yolk solution) to 100.0 cc. of the bouillon, prepared as before but containing no peptone.

Reference: Besredka and Jupille (1913 p. 1009).

1008. Stickel and Meyer's Meat Digest Solution

Constituents:

1. Water, tap	4000.0 cc.
2. Stomachs (hog)	400.0 g.
3. Liver, beef placenta or blood clots	400.0 g.
4. K_2HPO_4	8.0 g.

Preparation:

(1) Wash clean and mince finely 5 or more large pig's stomachs. Mince an equal amount of clean pig's or beef liver, cheap fat-free beef, placenta or blood clots.
(2) Mix 2, one of 3 and 40.0 g. of Baker's Chemical Co. HCl in 1 at 50°C. and keep at 50°C. for 18 to 24 hours.
(3) Make a Biuret and tryptophane test. When both are + the digest is yellowish green and contains very little undigested debris.
(4) Transfer to large bottles and steam for 10 minutes at 100°C. to stop digestion.
(5) Strain thru cotton or preferably store over night in the ice chest and decant after 24 hours.
(6) Warm (5) to 70°C. and neutralize with 2 N Na_2CO_3, to litmus.
(7) Filter into a flask.
(8) Add 8.0 g. K_2HPO_4.
(9) Adjust to desired reaction using litmus or preferably to a definite H-ion concentration (pH 7.0 to 7.5).
(10) Heat in steamer at 100° for 15 minutes.
(11) Correct reaction and filter thru paper.
(12) Distribute in receptacles used for cultures.

Sterilization: Sterilize at 100° for 30 minutes on 2 successive days or at 10 pounds pressure for 15 minutes.

Use: General inexpensive culture medium.

Variants:

(a) The authors prepared a medium as indicated from step (1) thru (6). The remainder of the preparation was as follows:
  (7) Sterilize at 10 pounds pressure for 15 minutes in the autoclave or for 30 minutes at 100°C. on 2 successive days.
  (8) Inoculate (7) with 1.0% of a 24 hour old broth culture of *B. saccharolyte* or *B. coli* and incubate for 12–18 hours at 37°C.
  (9) Steam for 20 minutes.

(10) Adjust to desired reaction.
(11) Add 0.2–0.4% K_2HPO_4 and 2.0% of purified talcum.
(12) Filter thru paper.
(13) Distribute for use.
(14) Sterilize at 100°C. for 30 minutes on 2 successive days or at 10 pounds pressure for 15 minutes.

(b) Park, Williams and Krumwiede omitted the K_2HPO_4.

References: Stickel and Meyer (1918 pp. 78, 79), Harvey (1921–22 p. 99), Park, Williams and Krumwiede (1924 p. 119).

1009. Frieber's Gelatin Digest Solution

Constituents:

1. Water	2000.0 cc.
2. Gelatin	20.0 g.
3. NaCl	5.0 g.
4. KH_2PO_4	2.0 g.
5. $MgSO_4$	0.2 g.
6. Tryptophane	0.3 g.

Preparation:

(1) Add 20.0 g. of gelatin, 10.0 cc. HCl and 2.0 g. Witte's peptone to 2 liters of water and allow to digest for several days.
(2) Neutralize to litmus by adding NaOH.
(3) Dilute (2) with an equal volume of water.
(4) Dissolve 3, 4, 5 and 6 in (3).
(5) Add 7.0 cc. of normal soda solution.

Sterilization: Not specified.

Use: Indol production.

Reference: Frieber (1921–22 p. 263).

1110. Frieber's Fibrin Digest Solution

Constituents:

1. Water.	
2. Fibrin.	
3. NaCl	0.5%

Preparation:

(1) Wash fibrin in running water until it is white. Press out the water. The fibrin containing about 25% dry material is placed in a flask so that the flask is about ⅓ full.
(2) Add to each liter flask about 3 to 5 grams pepsin powder and fill the flask with water.
(3) For each liter of material add 10.0 cc. of concentrated (1.19) HCl.
(4) Shake thoroly.
(5) Allow the flask to stand at room temperature or in the incubator. Shake thoroly several times a day.
(6) Add 5.0% HCl from time to time. Use congo red as an indicator (blue color).
(7) About 10 to 14 days are required for complete digestion of the fibrin. The reaction may be allowed to reach acid to litmus at this time.
(8) Allow the digest to stand undisturbed for 2 or 3 days.
(9) In order to obtain a fat-free digest, remove the bottom dark brown digest by means of a rubber tubing. The fat floats on the top surface.
(10) In order to obtain about a 1.0% peptone solution dilute the stock solution about ⅓ with water and add 5.0% NaOH solution to neutralize to litmus.
(11) A heavy flaky precipitate is formed. Do not add an excess of NaOH or the precipitate will dissolve.
(12) Boil and filter or allow the precipitate to settle. This gives a light yellowish 1.0% peptone solution. It is necessary to add 0.5% NaCl.
(13) Distribute in flasks.

Sterilization: Method not given.

Use: Substitute for commercial peptone.

Reference: Frieber (1921 p. 425).

1111. Bramigk's Peptic Tryptic Digest Solution

Constituents:

1. Water	4000.0 cc.
2. Blood clot	1000.0 g.
3. Stomach (hog)	2

Preparation:

(1) Prepare Bramigk's Blood Clot Digest solution, see medium 1004.
(2) Add $Ba(OH)_2$ to 1000.0 cc. of (1) until the reaction is only slightly acid.
(3) Boil to clarify.
(4) Add without filtration 5.0 cc. water free soda (strength solution not given), 5.0 cc. toluol, 5.0 cc. chloroform, and 2.0 g. pancreaten (Rhenania).
(5) Mix well and incubate at 37° for 120 hours, shaking occasionally. (Test for tryptophane using bromine

water.) Add HCl to stop the digestion.

Sterilization: Method not given.

Use: Indol production. Use this digest in 1.0 or 2.0% solution for indol reaction. Author reported that the addition of 0.1% of this digest gave equally as good growth when added to nutrient medium as did 1.0% Witte's peptone.

Reference: Bramigk (1921 p. 431).

1112. Jensen's Milk Digest Solution

Constituents:

1. Milk	1000.0 cc.

Preparation:

(1) To sterilized milk add 10.0 cc. of pure concentrated HCl per liter and 2.0 g. of a pure pepsin preparation.
(2) Incubate at 35–37° for 36 to 48 hours shaking often at first until the casein has precipitated and to dissolve the pepsin, and then only occasionally.
(3) Approximately neutralize the acid (acid hydrolyzes the lactose when heated) and heat in the autoclave at 115 to 120° for 10 minutes.
(4) Filter thru paper.
(5) Adjust the reaction (neutral to litmus).
(6) May be clarified with egg white if desired.

Sterilization: Sterilize in the usual manner.

Use: Cultivation of lactic acid bacteria.

Reference: Jensen (1898 p. 199).

1113. Stickel and Meyer's Trypsinized Blood Clot Solution

Constituents:

1. Water, tap	1000.0 cc.
2. Blood clots	500.0 g.
3. K_2HPO_4	2.0 g

Preparation:

(1) Obtain 10 liters fresh beef blood from the abattoir.
(2) Decant and store the serum (which has separated on standing) in a refrigerator.
(3) Weigh the blood clots and mix 500.0 g. with 1 liter tap water.
(4) Place the mixture in an enameled pot, bring slowly to a boil, and boil slowly for 5 minutes, stirring constantly.
(5) Strain fluid thru cheese cloth and pass the residue thru a fruit press, cool to 37°C.
(6) Make the thick brownish fluid slightly alkaline to litmus.
(7) Add 1.0% pancreatic extract and incubate at 37° for 5, 24, 48 hours.
(8) When the process is sufficiently advanced, render slightly acid with glacial acetic acid and boil slowly for 15 minutes.
(9) Either filter or decant the clear fluid which results on placing the digest over night in a cool place.
(10) Adjust the reaction as desired.
(11) Dissolve 3 in (10).
(12) Heat for 15–30 minutes in the steamer at 100°C.
(13) Filter again if necessary.
(14) Clear (13) by adding 5–10.0% of the decanted beef serum. Steam 45–60 minutes.
(15) Remove (14) from steamer and allow clot to form as a compact mass. Decant, or better, centrifuge the medium to remove the clot.

Sterilization: Sterilize at 100° for 30 minutes on each of 2 successive days.

Use: Author reported that this medium was excellent for primary isolation of highly parasitic organisms.

Variants: Knorr prepared a similar medium as follows:

(1) Mix 1 kilo of blood clots with 1500.0 cc. water, and boil for 1 hour on an open flame. Stir often.
(2) Press the fluid thru a filtering cloth and run the blood clot thru a meat grinding machine.
(3) Add the ground blood clot to the fluid. Stir.
(4) Add 1.0 to 2.0 g. pancreatin and chloroform and digest for 6 to 7 days at room temperature. Mix the chocolate brown liquid often.
(5) Acidify to stop the digestion.
(6) Filter thru paper.
(7) Add enough water to the residue to make a 4000.0 cc. stock solution.
(8) Add 100.0 g. of rock salt (cattle salt) and 15.0 g. potassium phosphate.
(9) Filter. This is the stock solution. It gives a tryptophane reaction.

(10) When ready for use, use in 40.0% strength.
(11) Sterilization of stock solution not specified.

References: Stickel and Meyer (1918 p. 81), Knorr (1921 p. 598), Harvey (1921–22 p. 117), Klimmer (1923 p. 176).

1114. Spray's Blood Clot Digest Solution

Constituents:
1. Water.
2. Blood clot.
3. Na_2CO_3.

Preparation:
(1) Remove the serum from the blood clot and allow to drain.
(2) Pass the clot thru a wire gauze.
(3) Boil and divide finely again.
(4) Place one liter of this semi fluid material in a two liter flask.
(5) Add 20.0 g. anhydrous Na_2CO_3, 5.0 g. trypsin, and 15.0 to 20.0 cc. chloroform.
(6) Incubate for 15 days with a second addition of 3.0 g. trypsin on the tenth day. Chloroform may be added on the fifth and tenth days.
(7) Make strongly acid by the addition of 50.0 to 75.0 cc. HCl.
(8) Steam in the water bath to drive off the chloroform.
(9) Adjust to pH = 7.4 to 7.5.
(10) Tube.

Sterilization: Sterilize in the autoclave.

Use: The author reported that this blood clot digest may be added in from 5.0 to 10.0% to melted North's or other agar for growth of *H. influenzae*, pneumococcus and streptococcus. When sodium oleate is added *H. influenzae* grows very vigorously.

Reference: Spray (1927 p. 14).

1115. Löffl's Trypsinized Blood Solution

Constituents:
1. Water.
2. Blood.

Preparation:
(1) Allow 9 liters of blood to flow into a liter of 0.8% ammonium oxalate.
(2) Mix well.
(3) Centrifuge at 3000 revolutions per minute.
(4) This yields about 6 liters of plasma.
(5) Precipitate out the oxalate by the addition of calcium acetate.
(6) Add 40.0 g. trypsin to the plasma and allow it to act for 8 days.
(7) At the end of this time, boil for a few minutes, allow to stand and filter.

Sterilization: Method not given.

Use: Inexpensive medium. Author reported that this medium contains about 8.0% nutrient materials and should be diluted 1:3 before use.

Reference: Löffl (1915–16 p. 109).

1116. Gordon et al. Trypsinized Heart Solution (Tanner)

Constituents:

1. Water	1000.0 cc.
2. Heart, beef	500.0 g.

Preparation:
(1) Free fresh bullock's heart from fat and blood vessels.
(2) Mince (1) very finely and weigh.
(3) To each 0.5 kilo add 1 liter of water, and make faintly alkaline to litmus by the addition of 20.0% KOH solution.
(4) Heat slowly to 75 to 80°C. for 5 minutes.
(5) Cool to 37°C. and add 1.0% of liquid trypsine comp., and keep at 37° for 2.5 to 3 hours.
(6) When trypsinizing is finished test for peptone with copper sulphate and KOH (Method not given).
(7) Render slightly acid by the addition of glacial acetic acid.
(8) Bring slowly to boil and boil for 25 minutes.
(9) Leave over night in a cool place.
(10) Siphon off the clear liquid in the morning.
(11) Make faintly alkaline to litmus.

Sterilization: Sterilize in an autoclave at 118° for one hour on each of two days.

Use: General culture medium.

Reference: Tanner (1919 p. 45).

1117. Harvey's Trypsinized Heart Solution

Constituents:

1. Water	400.0 cc.
2. Heart, ox	1
3. NaCl	1.0 g.
4. $CaCl_2$	0.5 g.

Preparation:

(1) Mince finely an average sized ox heart.
(2) Add to 400.0 cc. tap water.
(3) Heat slowly to 75°C.
(4) Allow to cool to 45°C.
(5) Add trypsin solution to 1 per cent.
NOTE: e.g., Liq. trypsin Co. (A (and H)).
(6) Place in incubator $2\frac{1}{2}$ hours.
(7) Test for peptone by the Biuret test.
NOTE: Add 1.0 cc. 5 per cent copper sulphate to 5.0 cc. trypsin digest, followed by 5.0 cc. N/1 potassium hydroxide. Note the color change. If the color is pink, peptonization is complete; if bluish purple incomplete.
(8) Make faintly acid to litmus with 4 per cent acetic acid.
(9) Boil 15 minutes.
(10) Allow the solid matter to settle.
NOTE: Or simply strain thru cloth.
(11) Pour off the supernatant fluid.
(12) Add 1.0 g. sodium chloride and 0.5 g. calcium chloride.
(13) Make faintly alkaline to litmus.
(14) Steam 45 minutes.
(15) Bring up to original volume.
(16) Adjust the reaction.
(17) Steam 30 minutes.
(18) Clarify by the addition of egg albumin.
(19) Tube.

Sterilization: Sterilize in the autoclave or steamer.

Use: General culture medium. Author reported good growth of meningococci and similar organisms when pea extract or serum was added.

Variants: The author gives the following variants:

(a) Add 25.0% of sterile rabbit or horse serum.
(b) Add 5.0% of a sterile pea extract prepared by steaming a mixture of 50.0 g. pea flour and 100.0 g. NaCl in 1000.0 cc. distilled water for 30 minutes, filtering thru paper and sterilizing.
(c) The medium was prepared as follows from horse heart, omitting the NaCl and $CaCl_2$.
(1) Mince finely fat-free horse heart.
(2) Add 500.0 g. to 1000.0 cc. water.
(3) Make faintly alkaline to litmus.
(4) Heat slowly to 75°C.
(5) Allow to cool to 45°C.
(6) Add trypsin solution to 1 per cent and 35.0 cc. chloroform.
(7) Place in a loose stoppered bottle.
(8) Keep 10 days at 37°C. with daily shaking.
NOTE: The reaction must be frequently tested and made faintly alkaline to litmus.
(9) Add, at the end of this time, trypsin solution again to 1 per cent.
(10) Keep a further period of 10 days at 37°C. without shaking:
NOTE: The reaction must be frequently tested and made faintly alkaline to litmus.
(11) Make faintly acid to litmus with 4 per cent acetic acid.
(12) Boil 15 minutes.
(13) Allow the solid matter to settle by placing in the ice chest over night.
(14) Pour off the supernatant fluid or filter thru well-wetted, thick, filter paper.
(15) Make the reaction 1.2 per cent acid to phenolphthalein.
(16) Steam 45 minutes.
(17) Filter.
(18) Sterilize in the steamer or autoclave.

Reference: Harvey (1921–22 p. 114).

1118. Distaso's Trypsinized Serum Solution

Constituents:

1. Distilled water	500.0 cc.
2. Serum (beef of sheep)	500.0 cc.

Preparation:

(1) Mix equal parts of water and sheep or beef serum.
(2) Digest sterile (1) for 24 hours at 60°C. with a pancreatic extract from a hog in the presence of chloroform. Activate the extract with an extract of the upper portions of the small intestines.
(3) Filter on paper.
(4) Tube.

Sterilization: Sterilize (1) at 120° for 15 minutes. Sterilize (4) method not given.

Use: General culture medium. Substitute for bouillon. Author stated that sugars might be added to this medium if desired. Harvey used a similar medium to demonstrate the indol test.

Variants:

(a) The author gave a more complete method of the preparation as follows:

(1) Chop the pancreas of a hog into small pieces and extract with chloroform in 9 times its volume of distilled water for 25 hours.
(2) Filter on glass wool, pressing the meat free from the liquid.
(3) Add ether to preserve the extract and store in a sealed flask.
(4) Mix beef or sheep serum with an equal volume of water.
(5) Boil (4) until it assumes a milky appearance.
(6) Mix 15 volumes of (3) with 100 volumes of (5) and digest over night at 60°C. on a salt water bath.
(7) Filter.
(8) Tube
(9) Sterilize at 120° for 15 minutes.

(b) Harvey mixed one part serum with 2 parts tap water and boiled until the mixture was milky. Then added 15.0 cc. of pancreatic extract to 100.0 cc. of the mixture and digested at 60°C. for 24 hours. Following filtration thru paper and distribution, the medium was sterilized by heating at 120°C. for 15 minutes. This medium was used for the detection of indol production.

(c) Harvey used equal parts serum and water boiled as above and added 10.0% pancreatic extract. After digestion at 60°C. over night, the medium was filtered thru thick filter paper, tubed and sterilized in the autoclave.

References: Distaso (1916 p. 600), (1917 p. 253), Harvey (1921–22 pp. 81, 117).

1119. Hottinger's Trypsinized Meat Solution (Klimmer)

Constituents:

1. Water.
2. Meat.
3. K_2HPO_4.
4. NaCl.
5. K_3PO_4.

Preparation:

(1) Add 1.5 liters of boiling water to 1000.0 g. of meat, cut in pieces the thickness of a finger.
(2) Apply heat until the mixture boils again.
(3) Remove the pieces of meat.
(4) Pass the meat thru a grinding machine.
(5) Place the liquid in a large mouthed flask, add 1.5 g. dehydrated soda and when the liquid is lukewarm add 3.0 g. pancreatin and 15–20.0 cc. chloroform (and toluol).
(6) Seal the flask and mix well.
(7) Add the ground meat (4) and shake once more.
(8) The meat should be thoroly covered with liquid. If not add water.
(9) Incubate for 5 days at 20° or 2 days at 37°. Shake daily.
(10) Acidify slightly by the addition of HCl.
(11) Filter thru paper.
(12) Wash the residue on the filter paper until the volume of the filtrate is 3 liters.
(13) Filter again after shaking thoroly.
(14) Boil for at least 10 minutes.
(15) Dilute to 10 to 50 liters.
(16) Prepare a saturated solution of crude salt (not purified) rich in calcium and magnesium.
(17) Dissolve 5.0 g. of K_2HPO_4 and small knife point of K_2HPO_4 in a little distilled water. Filter.
(18) Add (17) to 100.0 cc. of filtered (16).
(19) Add 20.0 cc. of (18) to each liter of (15).
(20) Filter.
(21) Boil.
(22) Make slightly alkaline.

Sterilization: Not specified.

Use: General culture medium. Klimmer reported that for bacteria producing pigment dilute the solution to 50 liters in step (15), for usual laboratory work to 30 liters and to 10 liters if an extremely good culture medium is desired.

Variants:

(a) Park, Williams and Krumwiede give the following method of preparation of the Hottinger medium. The phosphates are omitted.

(1) Free 750.0 g. meat from fascia and cut in finger-thick pieces.
(2) Drop (1), piece by piece, into 1500.0 g. of boiling water, stirring constantly.
(3) Boil up strongly and take from fire.
(4) Take out the meat and put thru a chopping machine.
(5) Cool the water to 37°C. and add 1.5 g. Na_2CO_3 per liter.
(6) Put the chopped meat in flasks (2 liter Erlenmeyer) 550.0 g. in each flask.
(7) Add (5) (the water) at 37°C. to the flask, filling them up to the narrow neck.
(8) Add 3.0 g. of pancreatin, 10.0 cc. chloroform and 10.0 cc. toluol to each flask.
(9) Cork tightly.
(10) Shake thoroly.
(11) Incubate at 37° over night.
(12) Shake the next morning and add more pancreatin unless the fluid shows a yellow color, and particles of meat look smaller, indicating digestion.
(13) Digest for 4 or 5 days at room temperature or for 2 or 3 days in the incubator. Shake the flasks each day. The meat should be in a finely divided mass, giving off a very offensive odor, when digestion is complete.
(14) The medium may be stored in the ice box, after acidifying to litmus by the addition of HCl, if necessary. (Hottinger's statement.)
(15) As soon as the digestion is complete decant the liquid thru cheese cloth. (This process gives better results according to the author.)
(16) Add an equal amount of water to the residue.
(17) Shake (16) thoroly.
(18) Allow to settle and again decant.
(19) Place the meat on the cheese cloth and allow to drain.
(20) Boil the filtrate a few minutes.
(21) Filter thru absorbent cotton and paper until clear.
(22) Autoclave at 15 pounds pressure for 30 minutes.
(23) Store as stock broth.
(24) Dilute the stock broth as desired for use. According to Hottinger it may be diluted 10, 20 or more times. Excellent results were obtained, however, by diluting 1 part stock broth with 1 part water.

(b) Park, Williams and Krumwiede prepared a similar medium as follows:
  (1) Add 300 to 500.0 g. of fat-free chopped meat to 1000.0 g. of water to which 0.4% Na_2CO_3 has been added.
  (2) Soak over night.
  (3) Heat at 80°C.
  (4) Cool to 38°C. and add 15.0 cc. of liquid trypsin.
  (5) Keep at 38°C. for 5 hours stirring frequently. If kept over night at this temperature add 10.0 cc. of toluol (or thymol crystals).
  (6) Add normal HCl to neutralize (indicator not specified).
  (7) Boil 7 minutes.
  (8) Strain.
  (9) Adjust the reaction.
  (10) Boil 30 minutes.
  (11) Filter.
  (12) Sterilize (method not given).

References: Klimmer (1923 p. 175), Park, Williams and Krumwiede (1924 pp. 118, 119).

1120. Peckham's Trypsinized Beef Solution

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.
3. NaCl	5.0 g.
4. Trypsin	4.0 g.

Preparation:

(1) Place 500.0 g. of finely chopped beef which is as old as can be obtained from the shops, in order that it be free from muscle sugar, in 500.0 cc. of water.
(2) Make slightly alkaline with sodium carbonate.
(3) Place in a water bath and raise temperature to 40°C. and add trypsin.
(4) After an hour the mixture must be again made alkaline with sodium carbonate.
(5) Allow to digest only from one to 1½ hours, or traces of indol may be detected.
(6) Boil and strain thru gauze.

(7) Filter thru wet filter paper when cold to remove fat.
(8) Add salt and enough water to bring the volume to 1 liter.
(9) Adjust so that 20 to 30.0 cc. of a decinormal NaOH solution will be required to bring one liter of the medium to the neutral point of phenolphthalein.
(10) Clarify and filter.

Sterilization: Method not given.

Use: Indol production.

Reference: Peckham (1897 p. 554).

1121. Harvey's Trypsinized Meat and Kidney Solution

Constituents:

1. Water	1000.0 cc.
2. Meat	500.0 g.
3. Kidney, rabbit	

Preparation:

(1) Mince finely, fat-free lean meat.
(2) Add 500.0 g. to 1000.0 cc. tap water.
(3) Heat the mixture 20 minutes at a temperature not exceeding 50°C.
(4) Skim off fat floating on the surface.
(5) Raise the temperature rapidly to boiling point.
(6) Boil 10 minutes.
(7) Make faintly alkaline to litmus.
(8) Add trypsin solution to 2 per cent.
(9) Place in incubator in an open vessel, 4 days.
(10) Filter thru well-wetted, thick filter paper.
(11) Bring the volume to 1000.0 cc. by the addition of water.
(12) Make neutral to phenolphthalein at room temperature.
(13) Store the sterile medium in sterile flasks under layer of paraffin to which sodium formate has been added to the extent of 1 per cent of the total volume of the medium.
(14) Test the sterility of the medium before use by anaerobic culture. Incubate at least 7 days.
(15) Add before use 1/16th part of fresh sterile rabbit kidney to 5.0 cc. medium. With kidney added, incubation may be aerobic. Use as soon as possible after the addition of the kidney.

Sterilization: Filter (12) thru a Berkefeld and a Doulton candle in series.

Use: Cultivation of *B. tetani* and other anaerobic organisms. Author reported that *B. sporogenes* was inhibited.

Variants: Harvey prepared a similar medium as follows:

(1) Mince finely fat-free beef or rabbit flesh.
(2) Add 500.0 g. to 1000.0 cc. water containing 5.0 g. sodium carbonate.
(3) Place in incubator 20 hours.
(4) Make faintly alkaline to litmus.
(5) Add trypsin solution to 2 per cent.
(6) Place in the incubator for a further 20 hours.
(7) Filter thru well-wetted, thick filter paper.
(8) Make faintly acid to litmus.
(9) Steam or boil 45 minutes.
(10) Filter thru well-wetted, thick filter paper.
(11) Bring the volume up to 1000.0 cc.
(12) Make neutral to phenolphthalein.
(13) Steam 30 minutes.
(14) Filter, while hot, thru well-wetted thick filter paper. Occasionally it may be necessary to filter thru a Doulton candle.
(15) Distribute the filtrate into test tubes.
(16) Add just before use 1/16th part of fresh sterile rabbit kidney and use as soon as possible after the addition of the kidney.

Reference: Harvey (1921–22 p. 115).

1122. Celozzi's Placenta Digest Blood Solution

Constituents:

1. Ringer-Locke solution	1000.0 cc.
2. Placenta	500.0 g.
3. Glucose (0.5%)	5.0 g.
4. Glycerol (2.0%)	20.0 g.
5. Blood	

Preparation:

(1) Thoroly wash fresh placenta and pass thru a meat chopper.
(2) Mix two parts by weight of Ringer-Locke solution with one part (1).
(3) Adjust the reaction to that of human blood and add 1.0% pancreatin and 0.5% chloroform.

(4) Digest at 40 to 42°C. for 24 hours.
(5) Filter thru cloth and paper, and adjust the reaction to the correct alkalinity.
(6) Steam for one hour, filter and add 0.5% glucose and 2.0% glycerol. (Agar and gelatin may be prepared from this medium.)
(7) Add 3 drops of blood to supply vitamines just before use.

Sterilization: Method not given.

Use: Used in human microparasitology. The addition of blood may be unnecessary with subcultures. Author claimed good results with anaerobes as well as aerobes.

Reference: Celozzi (1918 p. 291). Taken from (1919 p. 147).

1123. Douglas Trypsin Broth (Hartley)

Constituents:

1. Water	250.0 cc.
2. Meat, horse	150.0 g.
3. Na_2CO_3 (0.8% soln.)	250.0 cc.

Preparation:
(1) Mix 150.0 g. of lean minced horse meat with 250.0 cc. tap water and heat at 80°C. in a steamer.
(2) Add 250.0 cc. of an 0.8% Na_2CO_3 (anhydrous) and cool to 45°C.
(3) Add 5.0 cc. of chloroform and 5.0 cc. of Cole and Onslow's pancreatic extract.
(4) Incubate at 37°C. for 6 hours shaking frequently.
(5) Add 40.0 cc. normal HCl and heat in the steamer for 30 minutes.
(6) Cool and filter.
(7) Adjust to pH 8.0.
(8) Distribute as desired.

Sterilization: Pass steam thru the autoclave for one hour then raise the pressure slowly to 10 pounds and turn off the steam. For sterilization of larger quantities (one liter in a flask) maintain the pressure at 10 pounds for 30 minutes.

Use: Preparation of diphtheria toxin.

Reference: Hartley (1922 p. 482).

1124. Harvey's Basal Trypsinized Casein Solution

Constituents:

1. Water	1000.0 cc.
2. Casein	100.0 g.
3. NaCl	2.5 g.
4. $CaCl_2$	0.125 g.

Preparation:
(1) Add 100.0 g. commercial casein to 500.0 cc. boiling water containing 0.8% anhydrous Na_2CO_3.
(2) Raise the temperature to the boiling point.
(3) Add 500.0 cc. of cold water.
(4) Make faintly alkaline to litmus.
(5) Cool to 45°C.
(6) Add 400.0 cc. of pancreatic extract.
(7) Incubate 4 hours at 37°C.
(8) Raise the temperature to the boiling point.
(9) Filter thru thick wet clean cloth.
(10) Filter thru thick wet filter paper.
(11) Bring up the volume to 1000.0 cc. by the addition of water.
(12) Add 2.5 g. NaCl and 0.125 g. $CaCl_2$.
(13) Adjust the reaction.
(14) Steam 30 minutes.
(15) Filter while hot thru paper.
(16) Disolve 1.0% of one of the added nutrients in sterile (15).
(17) Distribute to a depth of one inch in small test tubes containing fermentation tubes.
(18) Cover with 0.25 inch of liquid paraffin.
(19) Add 3 drops of egg albumin mixed with bouillon (see variant 848 for preparation) to each tube. (This serves as an indicator.)
(20) Place the tubes in boiling water for 20 minutes.

Sterilization: Sterilize (15) in the autoclave.

Use: Determine fermentation of sugars by anaerobic organisms.

Added nutrients: Author added 1.0% glucose or any other desired carbohydrate, alcohol, etc.

Reference: Harvey (1921–22 p. 111).

1125. Teruuchi and Hida's Trypsinized Casein Solution

Constituents:

1. Water	1000.0 cc.
2. Casein	100.0 g.
3. NaCl (0.5%)	5.0 g.

Preparation:
(1) Dissolve 100.0 g. of pure casein in 1 liter of 0.8% Na_2CO_3 (anhydrous).
(2) Add 5.0 to 10.0 g. of pancreatin (Gehe and Co.) and shake with chloroform.

(3) Place for 3 to 5 days in the incubator, shaking occasionally.
(4) Each day test the mixture for tryptophane, by adding a few drops of acetic acid to a sample and then adding bromine water.
(5) When the tryptophane content has reached the maximum and the tyrosin has separated out as white clumps, heat the fluid for a short time at 80°C.
(6) Filter and neutralize with a few cc. of HCl.
(7) Heat moderately in a vacuum if possible and evaporate to a syrupy thickness. (Possibly may be filtered again.)
(8) Place in a mortar and knead it with alcohol (amount not specified).
(9) Dry in a vacuum desiccator.
(10) This yields a light yellow colored powder, which dissolves quite easily and clear in water. The yield is nearly quantitative.
(11) Add 0.5% NaCl and 1.0% Na_2CO_3 to a 5.0% watery solution of (9).

Sterilization: Not specified.

Use: Enrichment of cholera vibrio, toxin production by *Proteus vulgaris* and general culture medium.

Variants:

(a) Berthelot prepared a pancreatic casein digest, and dissolved 20.0 g. of it in a liter of water. The medium was sterilized with ether, method not given, and used to produce toxin by *Proteus vulgaris*.

(b) Harvey prepared a "tryptamine" medium as follows:
 (1) Prepare a suspension in a well stoppered bottle: casein 1; distilled water 10.
 (2) Shake well to break up clumps.
 (3) Adjust the reaction if necessary with the help of cresol red.
  NOTE: The optimum reaction for the tryptic digestion of casein is about pH = 8.1 at which point cresol red indicator solution gives a reddish violet color and phenolphthalein remains colorless.
 (4) Add per liter: Pancreatic extract 60.0 cc., toluol 5.0 cc.
 (5) Shake to mix.
 (6) Digest at 39°C. for 10 days, with daily shaking and addition of more toluol if necessary.
 (7) Add per liter, 100.0 cc. 7.5 per cent hydrochloric acid.
 (8) Steam 20 minutes.
 (9) Filter thru well-wetted, thick filter paper.
 (10) Make the reaction nearly neutral to litmus with 5% sodium hydroxide.
 (11) Preserve as stock tryptic broth or stock "tryptamine."
 (12) Dilute for use—"tryptamine" 1: water 2 = tryptamine bouillon.

(c) Harvey prepared a similar medium as follows:
 (1) Add very gradually 200.0 g. casein to 1000.0 cc. boiling water containing 20.0 g. anhydrous sodium carbonate.
 (2) Allow to cool to 45°C.
 (3) Add pancreatin 3.0 g. or pancreatic extract 50.0 cc., chloroform 15.0 cc.
 (4) Place in incubator 5 days, shaking vigorously each day to break up clumps.
 (5) Add again pancreatin 3.0 g. or pancreatic extract 50.0 cc.
 (6) Place in incubator again for 10 days.
 (7) Add 400.0 cc. N/1 hydrochloric acid.
  NOTE: Or 400.0 cc. pure concentrated hydrochloric acid diluted with 350.0 cc. water.
 (8) Steam 30 minutes.
 (9) Filter, while hot, thru well-wetted, thick filter paper.
 (10) Add 120.0 cc. N/1 sodium hydroxide to the filtrate.
 (11) Adjust reaction.
 (12) Dilute for use 1/3 with 0.5 per cent sodium chloride.
 (13) Sterilize in the autoclave or steamer.

References: Teruuchi and Hida (1912 p. 572), Berthelot (1914 p. 916), Harvey (1921–22 p. 116).

1126. Zipfel's Trypsinized Casein Solution

Constituents:
1. Distilled water............. 1000.0 cc.

2. Plasmon (sodium caseinate).	500.0 g.
3. KH_2PO_4	5.0 g.
4. Magnesium phosphate	0.3 g.
5. Ammonium lactate	

Preparation:

(1) Mix 500.0 g. of "plasmon" (sodium caseinate) with $2\frac{1}{2}$ liters of lukewarm 2.0% Na_2CO_3 solution.
(2) Add $2\frac{1}{2}$ liters of distilled water.
(3) Allow to soak and place in a large flask.
(4) Soak 10.0 g. of trypsin in a little water and add to (3).
(5) Place a layer of toluol about 1 cm. high to keep down bacterial growth.
(6) Place at 37°C. for several days shaking occasionally.
(7) From time to time test for tryptophane with acetic acid and bromine water. The maximum amount of tryptophane is reached after about 10 to 20 days.
(8) Remove the toluol and heat to 80°C. to coagulate any undigested albumin.
(9) Add 100.0 g. talcum and shake thoroly.
(10) Allow to stand several hours until it is rather clear.
(11) Tyrosin and some cystin will settle out during the cooling.
(12) Filter.
(13) Add H_2SO_4 to the clear filtrate until it is 5.0% H_2SO_4.
(14) Add a 10.0% solution of $HgSO_4$ in 5% H_2SO_4 to (13). A lemon colored precipitate of a union of $HgSO_4$ and tryptophane is formed.
(15) Allow to stand for 24 hours and filter. Test the filtrate to see that it is free from tryptophane by adding more $HgSO_4$ to it.
(16) Wash the precipitate with 5.0% H_2SO_4 until the wash water no longer gives a red coloration with Millon's reagent.
(17) Add the damp precipitate to 500.0 g. of distilled water.
(18) Decompose the precipitate by heating and pass in H_2S.
(19) Pass in CO_2 to drive out the excess of H_2S.
(20) The liquid contains tryptophane and cystin while the precipitate contains HgS.
(21) Filter and add H_2SO_4 to the filtrate so that it is 5.0% H_2SO_4.
(22) To separate the cystin, precipitate fractionally with $HgSO_4$ in that one adds first $HgSO_4$, carefully, until just a conglomerate precipitate of cystin mercury sulphate is formed.
(23) Allow to stand a few hours and filter.
(24) Precipitate again with $HgSO_4$.
(25) Allow to stand, filter, wash with 5.0% H_2SO_4 as in (16) and add the moist precipitate to distilled water.
(26) Decompose the precipitate with H_2S as in (18) and (19).
(27) Filter and evaporate to dryness.
(28) This brown colored mass may be crystallized in hot water several times or pure tryptophane may be obtained.
(29) To obtain pure tryptophane add lead carbonate (about 10.0% of the used albumin, therefore about 50.0 g.) to the filtrate (27) and heat for about 30 minutes in a water bath. Ammonia is given off.
(30) After cooling precipitate the lead by passing thru H_2S.
(31) Filter and evaporate the filtrate to dryness.
(32) After several crystallizations with dilute alcohol one obtains pure silvery rectangular platelets of tryptophane.
(33) Dissolve 0.3 g. of (32), 5.0 g. of secondary potassium phosphate, 0.3 g. of Magnesium phosphate and ammonium lactate (amount not given) in 1 liter of distilled water.
(34) Distribute in 10.0 cc. lots in tubes.

Sterilization: Sterilize on 2 successive days in a steam sterilizer.

Use: Indol production. Preparation of pure tryptophane.

Variants: The author added glucose and glycerol, amounts not given.

Reference: Zippel (1912–13 p. 572).

1127. Bacto Tryptophane Broth
(Dehydrated)

Constituents:

1. Distilled water	1000.0 cc.
2. Hydrolyzed casein 1.0%	10.0 g.
(Bacto tryptophane broth dehydrated)	

Preparation:

(1) Prepare a 1.0% solution of Bacto

Tryptophane Broth in hot distilled water.

(2) If sterilized at 15 pounds pressure for 20 minutes pH = 6.8±.

Sterilization: Sterilize at 15 pounds pressure for 20 minutes.

Use: Indol production. Medium permits a strong positive indol test in 12 hours.

Reference: Digestive Ferments Co. (1925 p. 13).

1128. Bacto Peptonized Milk (Dehydrated)

Constituents:

1. Distilled water	1000.0 cc.
2. Bacto Peptonized Milk (Dehydrated)	15.0 g.

Preparation:

(1) Dissolve 15.0 g. Bacto Peptonized Milk (Dehydrated) in 1000.0 cc. distilled water.

(2) If sterilized for 20 minutes at 15 pounds pressure pH = 6.5±.

Sterilization: Sterilize at 15 pounds pressure for 15 minutes.

Use: Cultivation of lacto-bacillus group.

Variants: Add 0.1% Bacto agar to medium for maintaining stock cultures.

Reference: Digestive Ferments Co. (1925 p. 14).

1129. Mueller's Trypsinized Casein Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl (1.0%)	10.0 g.
3. $MgSO_4$ (0.04%)	0.4 g.
4. $CaCl_2$ (0.02%)	0.2 g.
5. K_2HPO_4 (0.2%)	2.0 g.
6. Glucose (0.2%)	2.0 g.
7. Phenol red (0.02% solution).	80.0 cc.
8. Casein	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Digest a solution of commercial casein with Fairchild's trypsin at 37°C. for 2 weeks.

(3) Boil.

(4) Filter. Twelve cubic centimeters of the filtrate represents about 0.5 g. of casein. This is an impure casein digest.

(5) Mix 12.0 cc. of (4) with 13.0 cc. of water and add 25.0 cc. of (1).

(6) Adjust to pH = 8.0.

Sterilization: Sterilize in autoclave at 10 pounds for 10 minutes.

Use: To study requirements of streptococci and pneumococci. Author reported that the organisms grew in impure casein medium and not in pure casein.

Variants: The author prepared a pure casein digest by precipitating a solution of commercial casein in Na_2CO_3 three times by acetic acid. Then washed precipitate in alcohol and ether. Treat this purified casein as in steps (2), (3), (4) and (5) above.

Reference: Mueller (1922 pp. 318, 319).

1130. Cole and Onslow's Trypsinized Casein Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Casein	200.0 g.
3. NaCl (0.5% soln.)	2000.0 cc.

Preparation:

(1) Obtain the fresh pancreas of a pig, free it from fat as far as possible and weigh.

(2) Mince (1) finely and add 3 times its weight of distilled water, and its own weight of strong alcohol.

(3) Shake the mixture well in a large bottle, and allow to stand for 3 days at room temperature, shaking occasionally.

(4) Strain thru muslin and filter thru a large folded filter paper.

(5) Measure the filtrate and add 1.0 cc. of concentrated HCl for each liter of the filtrate.

(6) Allow to stand for a few days and filter off the sediment.

(7) The filtrate may be stored in a stoppered bottle, without the addition of an antiseptic. If the extract is to be used at once it is not necessary to add the HCl.

(8) Add 20.0 g. of anhydrous Na_2CO_3 to one liter of tap water, and boil in a 2 liter flask or large evaporating basin.

(9) When (8) is boiling, transfer the vessel to a boiling water bath, and gradually dust in 200.0 g. of casein (Laitproto #6 for bacteriological purposes from Casein Limited). Stir well to avoid the formation of lumps.

(10) Transfer to a Winchester quart and wash out the mixing vessel with a liter of cold tap water. Add this wash water to the casein solution.
(11) Cool to 40°C. and add 50.0 cc. of (7) and 15.0 cc. of chloroform.
(12) Shake well, stopper with a loose cork, and incubate at 37° to 40°C. Shake the bottle each day to break up lumps.
(13) Add 50.0 cc. more of (7) after 5 days and allow to stand for 10 more days. The material is digested 15 days in all.
(14) Shake well and transfer to a large 3 liter flask, add 400.0 cc. of normal HCl (or 40.0 cc. of pure concentrated HCl diluted with 360.0 cc. water). Shake, steam for 30 minutes and filter.
(15) Add 120.0 cc. of normal NaOH.
(16) Adjust to pH = 7.35.
(17) This is a stock broth solution.
(18) To prepare tryptic broth dilute (17) with twice its volume of 0.5% NaCl. Adjust the reaction to pH = 7.35.

Sterilization: Sterilize on 3 consecutive days rather than in the autoclave. (Details not given.)

Use: Substitute for peptone. General culture medium.

Variants: The author dissolved 2.0 g. of glucose or dulcitol in 40.0 cc. of a 0.04% phenol-sulphone-phthalein solution in a liter of the medium as prepared above. The medium was tubed in 3.0 cc. quantities and sterilized in steam on each of 3 successive days for 20 minutes each day.

Reference: Cole and Onslow (1916 pp. 10, 1012).

1131. Berman and Rettger's Trypsinized Casein Solution

Constituents:

1. Water	1000.0 cc.
2. Casein	
3. Beef extract	2.5 g.
4. NaCl	5.0 g.

Preparation:

(1) Digest a solution of casein with commercial trypsin at 45°C. for several hours.
(2) Dissolve 2.5 g. beef extract and 5.0 g. NaCl in 1000.0 cc. of (1).

Sterilization: Method not given.

Use: To study bacterial nutrition.

Reference: Berman and Rettger (1918 pp. 383, 384).

1132. Norris' Trypsinized Caseinogen Solution

Constituents:

1. Water	1000.0 cc.
2. Caseinogen	100.0 g.

Preparation:

(1) Digest 100.0 g. (10.0%) caseinogen in an 0.8% aqueous solution of washing soda with a 0.5% pancreatic extract at 37°C. for 24 hours.
(2) Concentrate the products of digestion on the water bath yielding a paste of the consistency and color of Liebig's meat extract.
(3) Add 100.0 g. (10.0%) of 2 to 100.0 cc. tap water.
(4) Adjust the reaction to +1.0.

Sterilization: Method not given.

Use: General culture medium. The author suggested that the term "trypsinoid" be employed for the paste. Brewer's yeast and fresh yeast were treated in exactly the same manner but the casein product gave the best results.

Variants:

(a) The paste from (2) may be further dried in a desiccator over H_2SO_4, yielding a brittle resinous mass which may be powdered.
(b) The author dissolved (2) in physiological salt solution instead of tap water.

Reference: Norris (1920 p. 706).

1133. Cannon's Trypsinized Casein Solution (Norton and Sawyer)

Constituents:

1. Distilled water	1000.0 cc.
2. Casein (c.p.)	20.0 g.
3. Asparagin	5.0 g.
4. Ammonium lactate	5.0 g.
5. K_2HPO_4	2.0 g.
6. $MgSO_4$	0.2 g.

Preparation:

(1) Add 20.0 g. casein to 250.0 cc. distilled water and make alkaline to phenolphthalein with Na_2CO_3.
(2) Add 0.5 g. trypsin and allow to digest for 6 hours.

(3) Autoclave. (Time not specified.)
(4) Add asparagin, ammonium lactate, K_2HPO_4 and $MgSO_4$.
(5) Make up to 1 liter and adjust to +1 phenolphthalein.

Sterilization: Final sterilization not specified.

Use: To show production of indol by bacteria.

Reference: Norton and Sawyer (1921 p. 473).

1134. Duval and Harris' Tryptic Digest Solution

Constituents:

1. Serum, egg albumin, liver or placenta	200.0 cc.

Preparation:

(1) 200.0 cc. of fresh sterile human serum, egg albumin, or the equivalent in grams of liver or placenta tissue are placed in a flask and 20.0 cc. of a 1.0% physiological salt solution of sterile trypsin added.
(2) Digest for 5 days at 37°C., changing the reaction to neutral as occasion demands.
(3) Heat at 70°C. for one hour.

Sterilization: Filter thru a Berkefeld filter.

Use: Cultivation of leprosy bacilli. The author stated that the same materials might be autolyzed under sterile conditions at 40°C. for 2 weeks or hydrolyzed with a culture of Finkler and Prior's vibrio or some other bacterial proteolyzer for 24 to 36 hours, and then neutralized with normal NaOH. Sterilize as indicated above.

Reference: Duval and Harris (1911 p. 168).

1135. Harvey's Tryptic Digest Solution

Constituents:

1. Water	1000.0 cc.
2. Heart (ox), or placenta (human)	500.0 g.
3. K_2HPO_4	2.0 g.

Preparation:

(1) Free fresh hog pancreas from fat as far as possible.
(2) Mince finely and weigh.
(3) Add 3.0 cc. 0.5% hydrochloric acid for every gram of the minced pancreas.
(4) Stir the mixture at intervals for 30 minutes.
(5) Add 6.4 cc. 5.0% sodium hydroxide for every 100.0 cc. of 0.5% hydrochloric acid used.
(6) Stir well and filter thru folded filter paper.
(7) Shake up with a little toluol.
(8) Make the reaction less acid by the cautious addition of 10.0% sodium hydroxide.
(9) Store in a stoppered bottle in a cool, dark place.
(10) Mince finely ox heart or human placenta.
(11) Add 500.0 g. to 1000.0 cc. tap water.
(12) Make faintly alkaline to litmus.
(13) Heat slowly to 75°C. and maintain at this temperature 10 minutes.
(14) Cool to 37°C.
(15) Add 1.0 or 2.0% of (9) to (14).
(16) Keep at 37°C. for 4 hours. If the digestion is extended to 6 hours or longer it is necessary to add chloroform or toluene. Control the progress of digestion by Biuret and tryptophane tests.
(17) Make faintly acid to litmus with glacial acetic acid.
(18) Raise slowly to the boiling point.
(19) Boil gently 15 minutes.
(20) Filter.
(21) Adjust the reaction.
(22) Add 0.2% di-potassium phosphate.
(23) Steam 20 minutes.
(24) Clarify and filter.

Sterilization: Sterilize in the autoclave or steamer.

Use: General culture medium.

Reference: Harvey (1921–22 p. 114).

1136. Stickel and Meyer's Tryptic Digest Solution

Constituents:

1. Distilled water	1000.0 cc.
2. NaCl	5.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.2 g.
5. $CaHPO_4$	2.0 g.
6. K_2HPO_4	2.0 g.
7. Heart, beef or placenta, human	500.0 g.

Preparation:

(1) Remove fat from beef heart and chop finely, or rinse human placenta in water and pass thru a meat chopper.

(2) Mix 500.0 g. beef heart or human placenta with 1000.0 cc. water.
(3) Make faintly alkaline to litmus using N KOH or N Na_2CO_3.
(4) Heat slowly to 70–80° for 5–10 minutes.
(5) Cool to 37°C. and add 1.0% pancreatic extract or "Bacto" trypsin.
(6) Incubate at 37°C. for 2–5 hours.
(7) Control the progress of digestion by repeated Biuret and tryptophane tests. In case it is necessary to extend the digestion over a period of 6 hours add chloroform or toluene.
(8) When the process is sufficiently advanced, render slightly acid with glacial acetic acid and boil slowly for 15 minutes.
(9) Either filter or decant the clear fluid which results on placing the digest over night in a cool place.
(10) Adjust the reaction as desired.
(11) Dissolve 2, 3, 4, 5 and 8 in (10).
(12) Heat for 15–30 minutes in the steamer at 100°C.
(13) Filter again if necessary.

Sterilization: Sterilize at 100°C. on 3 consecutive days if not to be used at once.

Use: General inexpensive culture medium.

Reference: Stickel and Meyer (1918 p. 80).

1137. Stickel and Meyer's Digest Solution

Constituents:

1. Water, tap	4000.0 cc.
2. Liver, placenta or blood clot	400.0 g.
3. Stomach, hog	400.0 g.
4. K_2HPO_4	8.0 g.

Preparation:

(1) Wash clean and mince fine 5 or more large pig's stomachs.
(2) Mince an equal amount of clean pig's or beef liver, cheap fat free beef, placenta or blood clots.
(3) Mix 400.0 g. of (1), 400.0 g. of one of (2) and 40.0 g. of Baker Chemical Co. HCl in 1000.0 cc. of tap water at 50°C.
(4) Incubate at 50°C. for 18 to 24 hours.
(5) Make a Biuret and tryptophane test. When both are + the digest is yellowish green and contains very little undigested debris.
(6) Transfer to large bottles and steam for 10 minutes at 100°C. to stop digestion.
(7) Cool to 80°C. and make faintly alkaline to litmus using 2N KOH or 2 normal Na_2CO_3.
(8) Cool to 37°C. and add 1.0% pancreatic extract or "Bacto" trypsin.
(9) Keep the mixture at 37°C. for 3 to 10 hours depending on the reaction of the trypsin and the digestion desired. Control the process by repeated tests for tryptophane.
(10) When trypsinizing is sufficiently advanced render reaction slightly acid with glacial acetic acid, and bring slowly to boiling point for 10 minutes.
(11) Filter thru paper or keep in cool place over night and decant the clear liquid in the morning.
(12) Add K_2HPO_4 and adjust the reaction faintly alkaline or to the desired H-ion concentration.
(13) Heat in steamer at 100° for 15 minutes.
(14) Correct reaction and filter thru paper.
(15) Distribute in receptacles used for culture.

Sterilization: Sterilize at 100°C. for 30 minutes on 2 successive days or at 10 pounds pressure for 15 minutes.

Use: General inexpensive culture medium.

Variants: The authors prepared a medium as indicated above steps (1) thru (7). The remainder of the preparation was as follows:

(8) Cool to 37°C add 1.0% pancreatic extract or 40.0 g. trypsin, 8.0 g. K_2HPO_4, 4.0 g. $CaCO_3$, and 1.0% of a 24 hour old broth culture of *B. saccharolyte*.
(9) Incubate at 37°C. for 12–18 hours and control the digestion by tryptophane tests and the removal of carbohydrates by the gas formation in fermentation tubes.
(10) When the digest is sugar free steam 15 minutes.
(11) Adjust to desired reaction and steam another 15 minutes.
(12) Filter thru paper.
(13) Distribute as desired.

(14) Sterilize at 100° for 30 minutes on 2 successive days or 10 pounds for 15 pounds in the autoclave.

References: Stickel and Meyer (1918 p. 80), Harvey (1921–22 p. 101).

1138. Frieber's Digest Extract Solution

Constituents:

1. Water	1000.0 cc.
2. Physiological salt solution	3000.0 cc.
3. Fibrin	
4. Beef extract, Liebig's	5.0 g.
5. NaCl	5.0 g.

Preparation:

(1) Prepare Frieber's Fibrin Digest Solution by digesting fibrin with pepsin and HCl (see medium 1110).

(2) Add 5.0 g. Liebig's beef extract, 5.0 g. NaCl and 7.0 cc. of normal soda solution to 1000.0 cc. of (1).

(3) Neutralize to litmus and boil.

(4) Distribute in a flask having a closely fitting glass stopper.

(5) Cool to 40°C.

(6) Add 0.2 g. Grübler's trypsin, 10.0 cc. chloroform and 5.0 cc. toluol.

(7) Shake thoroly and place in the incubator for 24 hours.

(8) Filter thru a damp filter.

(9) Dilute 1 part (8) with 3 parts physiological salt solution.

(10) Distribute in 5.0 cc. lots.

Sterilization: Sterilize in the steamer for one hour.

Use: To detect indol production.

Reference: Frieber (1921 p. 427).

1139. Davis and Ferry's Hydrolyzed Gliadin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Gliadin	20.0 g.
3. Tryptophane	0.4 g.
4. Tyrosine	0.75 g.
5. Cystine	0.2 g.
6. NaCl	2.5 g.
7. K_2HPO_4	1.5 g.
8. $MgSO_4$	0.25 g.
9. Gelatin	20.0 g.

Preparation:

(1) Hydrolyze gliadin with 25.0% H_2SO_4 for 24 hours on sand bath. Temperature not given.

(2) Add $Ba(OH)_2$ until alkaline. Filter.

(3) Exactly neutralize with 10.0% H_2SO_4 and test for absence of both Ba and SO_4 ions.

(4) Concentrate to thick syrup in vacuum.

(5) Dilute to a final solids content of 2.0% with distilled water.

(6) Treat gelatin in the same manner as gliadin (1) thru (5) above.

(7) Dissolve 3, 4, 5, 6, 7 and 8 in 500.0 cc. of (6).

(8) Mix equal parts (7) and (5).

(9) Adjust to pH from 8.0 to 8.2.

(10) Steam 15 minutes and check the reaction.

(11) Distribute as desired.

Sterilization: Heat at 115°C. for 20 minutes.

Use: Cultivation of *Bact. diphtheriae* for toxin production.

Reference: Davis and Ferry (1919 p. 232).

1140. Robinson and Rettger's Hydrolyzed Edestin Casein Solution

Constituents:

1. Water	1000.0 cc.
2. Casein	30.0 g.
3. Edestin	30.0 g.
4. Lactalbumin	30.0 g.
5. Dextrose	0.5 g.

Preparation:

(1) Boil 50.0 g. of casein with 10.0% HCl under a reflex condenser until the solution no longer responds to the Biuret test.

(2) Evaporate on the water bath until nearly all the HCl is removed.

(3) Neutralize the remaining acid with NaOH. This solution is of dark brown color and is known as "Casein C."

(4) Treat edestin and lactalbumin in exactly the same manner as casein as in steps (1) thru (3).

(5) Dissolve 3.0% (3), 3.0% edestin product and 3.0% lactalbumin product in water.

(6) Adjust the reaction to neutral to litmus.

(7) Distribute in 20.0 cc. lots in 150.0 cc. Erlenmeyer flasks.

Sterilization: Sterilize at 12 to 14 pounds pressure for 15 minutes.

Use: Cultivation of *B. diphtheriae* and study toxin production. Authors reported that the cultures were nearly non-toxic.

Variants: The authors added 0.1% sterile glucose or lactose to the sterile medium.

Reference: Robinson and Rettger (1917 p. 364).

SUBGROUP I-C. SECTION 15

Liquid media or basal solutions not containing digests; containing plant derivatives of unknown chemical composition.

A_1. Containing yeast derivatives.
B_1. Basal solutions; employed with the addition of other nutrients.
Henneberg's Basal Yeast Infusion Solution........................ 1141
B_2. Complete media.
C_1. Containing additional organic carbon of known chemical composition.
D_1. Additional carbon supplied as carbohydrates.
Heinemann's Glucose Yeast Infusion Solution.................... 1142
Gassner's Lactose Yeast Infusion Solution........................ 1143
Korf's Sucrose Yeast Infusion Solution............................ 1144
Böttger's Nitrate Yeast Infusion Solution........................ 1145
D_2. Additional carbon supplied as alcohols.
Janke's Alcohol Yeast Infusion Solution............................ 1146
Bertrand's Sorbitol Yeast Infusion Solution........................ 1147
C_2. Not containing additional organic carbon of known chemical composition.
Thoinot and Masselin's Yeast Infusion Solution.................... 1148
A_2. Containing bacteria or derivatives.
Stoklasa's Arabinose Azotobacter Solution........................ 1149
A_3. Containing fungi (fleshy) or their derivatives.
Lanken and Meyer's Fungus Infusion Solution.................... 1150
A_4. Containing peat, moss, etc., or their derivatives.
Ampola and Garino's Nitrate Peat Solution........................ 1151
Revis' Peat Infusion Solution...... 1152
Brussoff's Iron Peat Infusion Solution............................ 1153
Schmidt's Hydrolyzed Peat Solution 1154
Schmidt's Peat Solution............ 1155
A_5. Containing organs of flowering plants or their derivatives.
B_1. Leguminous plants specified.
C_1. Commercial extracts used.
Buchanan's Basal Legume Extract Solution........................ 1156
Buchanan's Salt Legume Extract Solution........................ 1157
C_2. Commercial extracts not used.
D_1. Leaves and stems employed.
Buchanan's Basal Clover Infusion Solution........................ 1158
Buchanan's Vetch Infusion Solution 1159
D_2. Flowers employed.
Wilhelmi's Clover Flower Infusion Solution........................ 1160
D_3. Pods employed.
Reed and Cooley's Bean Pod Infusion Solution.................... 1161
D_4. Seeds or their derivatives employed.
Mazé's Sucrose Bean Infusion Solution............................ 1162
DeRossi's Glucose Bean Infusion Solution........................ 1163
Kaufmann's Jequirity Infusion Solution............................ 1164
Tanner's Pea Flour Infusion Solution............................ 1165
Stutzer's Basal Legume Seed Infusion Solution.................... 1166
B_2.* Non-leguminous plants or their derivatives specified.
C_1. Tubers or roots employed.
D_1. Containing potato or derivatives.
Smith's Potato Infusion Broth.... 1167
Elsner's Hydrochinone Potato Infusion Solution.................... 1168
Robertson and Davis' Potato Infusion Solution.................... 1169
Lubinski's Glycerol Potato Infusion Solution........................ 1170
Berthelot's Vegetable Infusion Solution............................ 1171
Migula's Potato Juice............ 1172
Winogradsky's Gypsum Root Solution............................ 1173
D_2. Not containing potato or derivatives.
Tanner's Carrot Infusion Solution.. 1174
Robertson and Davis' Carrot Infusion Solution.................... 1175
C_2.† Leaves or stems employed.
D_1. Hay or straw.

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* See B_3 next page.

† See C_3 and C_4 next page.

Migula's Hay Infusion Solution.... 1176
Winogradsky's Ferric Hydroxide Hay Infusion Solution (Molisch). 1177
Jensen's Nitrate Straw Solution.... 1178
Wolbach and Binger's Glucose Hay Infusion Solution................ 1179
Sherman's Soil Hay Infusion Solution............................ 1180

D_2.* Cabbage.
Smith's Cabbage Infusion Solution. 1181
Conrad's Glucose Cabbage Infusion Solution........................ 1182
Will's Cabbage Juice............... 1183

D_3. Other leaves or stems.
Reed and Cooley's Basal Spinach Infusion Solution................ 1184
Sucksdorff's Tea Infusion.......... 1185
Bushnell's Asparagus Solution..... 1186
Tausz and Peter's Leaf Infusion Solution........................ 1187
Müller-Thurgaw's Grape Vine Infusion Solution.................... 1188
Malenkovic's Wood Infusion Solution............................ 1189
Olitsky's Tomato Infusion......... 1190

C_3. Fruits or their derivatives employed.

D_1. Extracts or infusions used.
Schardinger's Prune Solution...... 1191
Sucksdorff's Coffee Infusion........ 1192
Tanner's Fruit Infusion Solution... 1193
Turner's Quince Seed Infusion Solution........................ 1194

D_2. Fruit juices used.

E_1. Grapes and wines specified.
Müller-Thurgau and Osterwalder's Grape Juice...................... 1195
Schukow's Grape Must Solution.... 1196
Perold's Wine Medium............. 1197

E_2. Currants specified.
Bierberg's Currant Must Solution.. 1198
Müller's Currant Must Solution.... 1199

E_3. Other juices or mixture of juices specified.
Ottolenghi's Melon Juice Solution.. 1200
Bierberg's Cherry Must Solution.. 1201
Müller-Thurgau and Osterwalder's Pear Juice Solution.............. 1202
Giltner's Fermented Cider......... 1203
Zikes' Nitrate Fruit Must Solution. 1204
Schukow's Fruit Must Solution.... 1205

D_3. Fruit used.
Bachman's Mince Meat Medium.... 1206

C_4. Grains or their derivatives employed.

D_1. Extracts or infusions of grains (not including malts, beer worts, etc.).
Harvey's Wheat Flour Solution.... 1207
Omeliansky's Flax Stem Solution.. 1208
Speakman and Phillips' Maize Mash Solution........................ 1209

D_2. Malt extract, beer wort, beer, etc., employed.

E_1. Beers used.
Lafar's Beer Solution.............. 1210
Waterman's Sucrose Beer Solution. 1211

E_2. Malts used.
Würtz's Malt Infusion Solution... 1212
Beijerinck's Malt Wort Solution... 1213
Peklo's Malt Infusion Solution.... 1214
Bokorny's Malt Infusion Solution.. 1215
Bacto Malt Extract Broth (Dehydrated)......................... 1216
Reddish's Malt Extract Solution... 1217

E_3. Beer worts used.
Park, Williams and Krumwiede's Basic Beer Wort Solution........ 1218
Peklo's Beer Wort Solution........ 1219
Nakazawa's Beer Wort............. 1220
Peklo's Tartrate Beer Wort Solution 1221
Will's Asparagin Beer Wort Solution 1222

D_3. Juices of grains employed.
Owen's Cane Juice.................. 1223
Owen's Sulphured Cane Juice Solution............................ 1224
Sherman's Corn Juice.............. 1225

D_4. Silage employed.
Buchanan's Silage Infusion Solution 1226

B_3. A variety of plant materials specified.
Reith's Plant Infusion Solution.... 1227
Reith's Blood Cell Plant Infusion.. 1228

1141. Henneberg's Basal Yeast Infusion Solution

Constituents:
1. Yeast infusion (3.0%)...... 1000.0 cc.

Preparation:
(1) Preparation of 3.0% yeast infusion not given.
(2) Dissolve one of the added nutrients in (1).

Sterilization: Method not given.

Use: Cultivation of lactic acid bacteria. Other investigators have used similar media for the cultivation of a variety of bacteria.

Added nutrients and variants:
(a) The author added 20.0% glucose, maltose or sucrose to the basal solution.

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* See D_3.

(b) Henneberg prepared a 10.0% yeast infusion solution and added 2.0 to 5.0% of any desired carbohydrate, alcohol, etc.

(c) Bobilioff-Preisser added one of the following materials to yeast infusion:

dextrin
galactose
levulose
lactose
glucose
maltose
raffinose
sucrose

The media were sterilized in the steamer.

(d) Müller-Thurgau and Osterwalder prepared the basal solution as follows:

(1) Add water (amount not given) to 1000.0 g. pressed yeast and boil.
(2) Filter.
(3) Dilute the filtrate to 10 liters by the addition of water.
(4) Acidify by the addition of 1.0% malic acid. (Do not add acid here when adding an acid as a carbon source.)
(5) Distribute in 100.0 cc. lots.
(6) Sterilize (Method not given).
(7) Add one of the following materials

Glucose	2.0% or 4.0%
Levulose	1.5% or 4.0%
Galactose	4.0%
Sucrose	5.0% or 4.0%
Maltose	4.0%
Malic acid	4.5% or 2.0%
Potassium malate (acid)	10.0%
Ethyl malate	10.0%
Calcium malate (acid)	10.0%
Citric acid	2.2% or 4.7% or 2.0% or 4.0%
Lactose	4.0%
Raffinose	1.0%
l-arabinose	15.0%
Xylose	10.0%
a-methyl glucoside	2.0%
Glycerin	1.0%
ammonium malate	10.0%

(8) Sterilize once more (method not given).

The media were used to study fermentation by *Bacterium mannitopoeum*, *Bacterium intermedium*, *Bact. Gayoni* and mannitol bacteria from wine. The authors also used the basal solution without any additions.

(e) Besson prepared the medium as follows:

(1) Boil 100.0 g. of beer yeast in 1000.0 cc. of water.
(2) Filter thru paper.
(3) Add 5.0% of glucose or sucrose.
(4) Add a small amount of phosphoric acid and then add sufficient lime water to give a slightly alkaline reaction.
(5) Heat for 5 minutes at 116 to 117°C.
(6) Filter.
(7) Tube.
(8) Sterilize at 115°C.

References: Henneberg (1903 p. 8), Bobilioff-Preisser (1916 p. 387), Müller-Thurgau and Osterwalder (1918 p. 2), Besson (1920 p. 35).

1142. Heinemann's Glucose Yeast Infusion Solution

Constituents:

1. Water	2000.0 g.
2. Yeast	1.0 lb.
3. Dextrose (10.0 to 15.0%)	200.0 to 300.0 g.

Preparation:

(1) Boil one pound of pressed yeast or 1 liter of washed yeast with 2 liters of water for one hour.
(2) Filter the solution until clear.
(3) Do not adjust the reaction.
(4) Dissolve 10.0 to 15.0% dextrose in (3).

Sterilization: Sterilize in the Arnold on 3 consecutive days.

Use: General culture medium.

Reference: Heinemann (1905 p. 130).

1143. Gassner's Lactose Yeast Infusion Solution

Constituents:

1. Water	18,000.0 cc.
2. Yeast, Brewers	10,000.0 cc.
3. Lactose	0.3% or 0.5%

Preparation:

(1) Place about 10 liters of brewers yeast in a flask and wash with water. Allow to stand for 30 minutes and pour off the liquid.
(2) Repeat the washing process until the wash water is no longer brown but slightly turbid. Washing 5 times is usually sufficient.

(3) After pouring off the last water add 18 liters of water to the remaining washed yeast cells.
(4) Boil in the autoclave or steamer as in the preparation of meat bouillon.
(5) Allow to stand for a suitable length of time and remove the liquid from the sediment or filter thru filter paper.
(6) Add 0.3 or 0.5% lactose to the filtrate or supernatant fluid.
(7) Adjust to slightly alkaline to litmus.

Sterilization: Not specified.

Use: General inexpensive culture medium.

Variants: The author used the medium with constituents of double strength, with or without 0.5% NaCl.

Reference: Gassner (1916–17 p. 311).

1144. Korff's Sucrose Yeast Infusion Solution

Constituents:

1. Water............ 2000.0 to 3000.0 cc.
2. Yeast............ 1000.0 g.
3. Sucrose.

Preparation:

(1) Mix 1000.0 g. of yeast with water and decant. Continue this until the liquid is colorless and does not reduce Fehling's solution.
(2) Pour the yeast in a porcelain funnel provided with a sieve plate.
(3) Wash repeatedly with distilled water.
(4) Place the yeast in a large flask with 2 or 3 liters of water and boil for 30 minutes.
(5) Allow to settle.
(6) Filter.
(7) Boil the filtrate once more.
(8) Filter.
(9) Determine the specific weight and dilute to a suitable specific weight (1.0033 g.).
(10) Dissolve 1000.0 g. of white sugar candy in 500.0 g. distilled water by heating on the water bath.
(11) Filter while hot in the hot water funnel into a large porcelain dish.
(12) Add 2 liters of warm absolute alcohol, stirring continually.
(13) Pour the cool alcohol from the sugar and wash the sugar with absolute alcohol and then with ether.
(14) Dry at 60°C. until all traces of dampness of alcohol or ether have disappeared.
(15) Prepare a 10.0% solution of (14) in distilled water.
(16) Mix about 10.0% of (15) with 10.0% of (9).
(17) Distribute in 150.0 cc. lots in fermentation flasks.

Sterilization: Sterilize in streaming steam.

Use: To study fermentation by yeast. Waterman used a similar medium to study the inversion of sucrose by bacteria.

Variants:

(a) Syrée prepared a similar medium as follows:
  (1) Wash brewers yeast in a decantation funnel with distilled water until the wash water, to which some yeast has been added, boiled and filtered, will give no reaction with Fehling's solution.
  (2) Boil 1000.0 g. of the washed yeast with 2 liters distilled water for 2 hours.
  (3) Filter.
  (4) Boil the filtrate once more.
  (5) Filter.
  (6) Estimate the nitrogen content by Kjeldahl's method and dilute so that the nitrogen content is 0.0448%.
  (7) Weigh 100.0 g. of dried sucrose, prepared as indicated above. Step (10) thru (14) in a liter volumetric flask.
  (8) Pipette 100.0 cc. of (6) in the flask with the sugar.
  (9) Add distilled water to make 1.0 liter volume.
  (10) Distribute into 150.0 cc. lots, plug with cotton and gum, and sterilize in streaming steam.
  (11) Shake often during cooling to saturate the medium with air.

(b) Henneberg added 10.0% sucrose to a 1.0 to 15.0% yeast infusion solution. The reaction was made neutral with soda. The medium was used for the cultivation of lactic acid bacteria.

(c) Will added 6.0% sucrose to yeast water (preparation not given).

(d) Waterman added 4.0% sucrose to yeast water (preparation not given) to study the inversion of sucrose by

B. racens, *B. Pasteurianum*, *B. aceti*, *B. melanogenum* and *B. xylunum*. He reported that after 3 days generally there was little or no reaction with Fehling's solution.

References: Korff (1898 p. 532), Syrée (1899 p. 12), Henneberg (1903 p. 8), Will (1908 p. 387), Waterman (1913 p. 455).

1145. Böttger's Nitrate Yeast Infusion Solution

Constituents:

1. Yeast water	1000.0 cc.
2. Sucrose (10.0%)	100.0 g.
3. KNO_3 0.2, 0.5, 1.0, 2.0 or 4.0%	

Preparation:

(1) Preparation of dilute yeast water not specified.
(2) Add 10.0% cane sugar to (1).
(3) Add 0.2, 0.5, 1.0, 2.0 or 4.0% KNO_3 to (2).

Sterilization: Not specified.

Use: To study toxicity of nitrates. Author reported that the presence of nitrates had little effect.

Variant: Author used 0.17, 0.41, 0.83, 1.67 or 3.33% $NaNO_3$ instead of KNO_3.

Reference: Böttger (1921 p. 224).

1146. Janke's Alcohol Yeast Infusion Solution

Constituents:

1. Water	900.0 cc.
2. Yeast (pressed)	100.0 g.
3. Alcohol (3.0%)	30.0 cc.

Preparation:

(1) Boil 100.0 g. of pressed yeast with 900.0 cc. water.
(2) Filter.
(3) Add 3.0% by volume of absolute alcohol after sterilization.

Sterilization: Method of sterilization of (2) not given.

Use: Cultivation of acetic acid bacteria found in beers.

Reference: Janke (1916 p. 6).

1147. Bertrand's Sorbitol Yeast Infusion Solution

Constituents:

1. Yeast infusion	1000.0 cc.
2. KH_2PO_4	0.1 g.
3. Sodium phosphate	0.1 g.
4. $CaCl_2$	0.1 g.
5. $MgSO_4$	0.06 g.
6. Sorbitol (5.0%)	50.0 g.

Preparation:

(1) Preparation of yeast infusion not given. It should contain about 5.0 g. of dissolved material per liter.
(2) Dissolve 2, 3, 4 and 5 in (1).
(3) Add 5.0% sorbitol to (2).

Sterilization: Not specified.

Use: Production of sorbose by *Bacterium xylinum*, *Mycoderma aceti*.

Reference: Bertrand (1898 p. 397).

1148. Thoinot and Masselin's Yeast Infusion Solution

Constituents:

1. Water	1000.0 cc.
2. Yeast	100.0 g.

Preparation:

(1) Add 100.0 g. of yeast to 1000.0 cc. of water.
(2) Mix slowly.
(3) Boil in the autoclave or on a water bath.
(4) Make slightly alkaline by the addition of soda.
(5) Filter.
(6) Distribute as desired.

Sterilization: Sterilize in the autoclave at 115° for 15 minutes.

Use: General culture medium.

Variants:

(a) Henneberg used a 2.5, 5.0 or 10.0% yeast water solution but did not give the method of preparation. He cultivated lactic acid bacteria.
(b) Heinemann prepared the medium as follows:
  (1) Boil one pound of pressed yeast or one liter of washed yeast with 2 liters of water for one hour.
  (2) Neutralize to phenolphthalein.
  (3) Filter the solution until clear.
  (4) Sterilize in the Arnold on 3 consecutive days.
(c) Gassner prepared a similar medium as follows:
  (1) Place about 10 liters of brewers yeast in a flask and wash with water. Allow to stand for 30 minutes and pour off the liquid.
  (2) Repeat the washing process until the wash water is no longer brown

but slightly turbid. Washing 5 times is usually sufficient.

- (3) After pouring off the last water add 18 liters of water to the remaining washed yeast cells.
- (4) Boil in the autoclave or steamer as in the preparation of meat bouillon.
- (5) Allow to stand for a suitable length of time and remove the liquid from the sediment or filter thru filter paper.
- (6) Adjust the filtrate or supernatant fluid to a slight alkaline reaction using litmus as an indicator.
- (7) Sterilization not specified.

(d) Besson prepared the medium as follows:

- (1) Boil 100.0 g. of beer yeast in 1000.0 cc. of water.
- (2) Filter thru paper.
- (3) Distribute as desired.
- (4) The reaction is slightly acid, but may be made slightly alkaline if desired.
- (5) Sterilize at 115°C.

(e) Dopter and Sacquépée used a medium prepared in the following manner:

- (1) Dialyse yeast in 6 times their weight of water. (Time not given.)
- (2) Boil, stirring constantly.
- (3) Filter.
- (4) Distribute.
- (5) Sterilize.

(f) Harvey added 75.0 g. pressed yeast to 1000.0 cc. of water. Further preparation not given.

(g) Harvey prepared a medium as follows:

- (1) Add 100.0 g. yeast to 1000.0 cc. water.
- (2) Boil 10 minutes.
- (3) Filter thru well-wetted, thick filter paper.
- (4) Adjust the reaction.
- (5) Distribute into flasks or test tubes.
- (6) Sterilize in the autoclave or steamer.

(h) Harvey prepared a similar medium as follows:

- (1) Prepare: Baker's yeast 1; water 5.
- (2) Boil 20 minutes, with vigorous stirring.
- (3) Place in a tall glass vessel 24 hours.
- (4) Decant the supernatant fluid.
- (5) Make neutral to litmus.

(i) Klimmer used a medium prepared as follows:

- (1) Dilute 10 liters of Brewers yeast or beer yeast with 20 liters of water.
- (2) Allow to stand for 0.5 to 11 hours, remove the water and add fresh. Repeat the process until the wash water is slightly turbid.
- (3) Make up the volume of the yeast to 18 liters.
- (4) Boil in the autoclave or steamer.
- (5) Allow to settle.
- (6) Decant or filter.
- (7) Use the filtrate as meat peptone and peptone or nutrose.
- (8) The solution may be evaporated to dryness.
- (9) The filtrate may be used by the addition of 0.5% NaCl and making slightly alkaline.

(j) Cunningham prepared a medium as follows:

- (1) Place 1000.0 g. of fresh pressed yeast in a double walled pot and mix thoroly with 1000.0 cc. of water.
- (2) Steam for one hour.
- (3) Filter thru paper.
- (4) Tube in 5.0 cc. quantities.
- (5) Sterilize by intermittent steaming.

References: Thoinot and Masselin (1902 p. 29), Henneberg (1903 p. 8), Heinemann (1905 p. 130), Gassner (1916–17 p. 311), Besson (1920 p. 34), Dopter and Sacquépée (1921 p. 121), Harvey (1921–22 p. 120), Klimmer (1923 p. 170), Cunningham (1924 p. 103).

1149. Stoklasa's Arabinose Azotobacter Solution

Constituents:

1. Distilled water	1000.0 cc.
2. d-arabinose	1.0 g.
3. $MgCl_2$	0.5 g.
4. Iron sulphate	0.1 g.
5. Azotobacter culture.	

Preparation:

- (1) Dissolve 2, 3, and 4 in 1.
- (2) Distribute in 250.0 cc. lots in Fernbach fermentation tubes.
- (3) Adjustment of reaction not specified
- (4) Add an azotobacter membrane from mannitol culture to each flask.

Sterilization: Sterilize thoroly (method not given).

Use: Decomposition of organic nitrogen products by *Bacillus mycoides*.

Reference: Stoklasa (1911 p. 470).

1150. Lanken and Meyer's Fungus Infusion Solution

Constituents:

1. Water	1000.0 cc.
2. Fungus, dry	25.0 g.
3. NaCl	5.0 g.

Preparation:

(1) Gather the fungus in large amounts and remove all dirt from them.

(2) Run thru a meat grinding machine.

(3) Dry the chopped fungus in flat plates and then rub to a fine powder which is dried and stored.

(4) Add 25.0 g. of (3) to 1000.0 g. water and allow to stand at room temperature for 24 hours.

(5) Filter. The filtrate should be clear. If not, add a teaspoonful of filter cell, shake thoroly and filter again.

(6) Add 5.0 g. NaCl, and make alkaline to litmus by the addition of 10.0% soda solution standing for 20 minutes.

Sterilization: Sterilize on 3 successive days for ½ hour in the steamer.

Use: Substitute for meat extract medium. Authors used anthrax staphylococci, colon-typhoid group and pyocyaneus, and reported better growth than on media containing peptone. *Lactarius turpis, Mordschwamm, Lactarius turminosus white Reizker, Lactarius rufus Rübling* and *Lactarius vuleruis Wolfschwamm* were the fungi used.

Variants:

(a) Wiegert prepared a similar medium as follows:

  (1) Boil 50.0 g. dried fungus with 500.0 cc. water until the fungus is softened.

  (2) Add 2.0 g. of NaCl.

  (3) Evaporate until a thick extract is formed.

  (4) Add 1.0, 2.0 or 3.0% of this extract instead of meat extract in the preparation of nutrient media.

(b) Klimmer used 50.0 g. dried fungus instead of 25.0 g.

References: Lanken and Meyer (1921 p. 511), Wiegert (1922–23 p. 110), Klimmer (1923 p. 204).

1151. Ampola and Garino's Nitrate Peat Solution

Constituents:

1. Water	1000.0 cc.
2. Peat	20.0 g.
3. $NaNO_3$	3.2 g.

Preparation: (1) Mix 2 and 3 with 1.

Sterilization: Method not given.

Use: To study denitrification. Authors reported that the nitrates were not reduced.

Reference: Ampola and Garino (1897 p. 309).

1152. Revis' Peat Infusion Solution

Constituents:

1. Ammonium hydrate.
2. Peat.
3. $CaCO_3$.

Preparation:

(1) Exhaust peat with dilute ammonium hydrate (Details of method not given).

(2) Neutralize the alkaline fluid with phosphoric acid.

(3) Filter and tube.

(4) Add $CaCO_3$ (amount not given).

Sterilization: Not specified.

Use: To study stability of physiological properties.

Reference: Revis (1910 p. 175).

1153. Brussoff's Iron Peat Infusion Solution

Constituents:

1. Water.
2. Peat.
3. Iron.

Preparation:

(1) Pour water (amount not given) over finely divided peat in a beaker.

(2) Heat in the autoclave under 2 atmospheres pressure for 30 minutes.

(3) Pour off the dark brown extract and press the peat free from liquid.

(4) Distribute the extract in high cylindrical glasses to a depth of 4 or 5 cm.

(5) After adding a suitable amount (exact amount not specified) of water, cover the glasses with glass plate cover glasses.

(6) Add 2 or 3 pieces of sterile iron filings to sterile (5) under aseptic conditions.

Sterilization: Sterilize (5) in streaming steam for 6 hours. Sterilize 2 or 3 pieces of iron filings dry heat at 150° for one hour.

Use: Cultivation of *Ferribacterium duplex*. Author reported that after 5 or 10 days a slight yellow membrane was formed.

Reference: Brussoff (1916 p. 549).

1154. Schmidt's Hydrolyzed Peat Solution

Constituents:

1. Water	1000.0 cc.
2. Peat	20.0 g.
3. Salts.	

Preparation:

(1) Add 1000.0 g. of 0.3% HCl to 20.0 g. of peat and hydrolyze for 1 hour at 2.5 atmospheres pressure.

(2) Filter.

(3) Neutralize and add an excess of $CaCO_3$.

(4) Add the usual salts (amounts or kinds not given). The salts are to be nitrogen free.

Sterilization: Not specified.

Use: To study nitrogen assimilation by soil forms. Author reported that azotobacter formed as a scum on the surface of the medium in several days.

Reference: Schmidt (1920 p. 283).

1155. Schmidt's Peat Solution

Constituents:

1. Water	1000.0 cc.
2. NH_4Cl (0.25%)	2.5 g.
3. K_2HPO_4 (0.05%)	0.5 g.
4. $CaCO_3$ (2.0%)	20.0 g.
5. Peat	20.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add 20.0 g. of peat to (1).

Sterilization: Not specified.

Use: Cultivation of soil and horse manure forms. Azotobacter. Author reported that azotobacter formed a membrane on the surface after several days.

Variants: The author used moss instead of peat.

Reference: Schmidt (1920 p. 284).

1156. Buchanan's Basal Legume Extract Solution

Constituents:

1. Water	1000.0 cc.
2. Legume extract (Park Davis & Co.).	

Preparation:

(1) Boil 1000.0 cc. of one of Park Davis & Company's standard legume fluid extract until all the alcohol has evaporated. The legume extracts used were those of clover, tolu, catechu, physostigma, red clover blossom, glycyrrhiza, kino, baptisia, senna and scoparius.

(2) Make up to 1000.0 cc. by the addition of water.

(3) Filter.

(4) Prepare 10.0, 5.0, 1.0 and 0.1% solutions of these extracts with water. In case of the clover extract, do not dilute. In case of the glycyrrhiza prepare only 1.0 and 0.1% solutions.

(5) Add one of the added nutrients to extract solutions.

Sterilization: Sterilize for 20 minutes on each of 3 successive days in streaming steam.

Use: To study gum formation by *Bacillus radicicola*. The author reported that the extract of catechu, physostigma, kino and scoparius inhibited growth. The remaining legume extracts favored growth and gum production.

Added nutrients: The author added 2.0% sucrose or 1.0% maltose to the extracts prepared as indicated in step (4) above.

Reference: Buchanan (1909 p. 391).

1157. Buchanan's Salt Legume Extract Solution

Constituents:

1. Water	1000.0 cc.
2. Legume extract (Park Davis & Co.).	

Preparation:

(1) Boil 1000.0 cc. of one of Park Davis & Company's standard legume fluid extract until all the alcohol has evaporated. The legume extracts used were those of clover, tolu, catechu, physostigma, red clover blossom, glycyrrhiza, kino, baptisia, senna and scoparius.

(2) Make up to 1000.0 cc. by the addition of water.
(3) Filter.
(4) Heat distilled water containing 0.2% KH_2PO_4 and 0.01% $MgSO_4$ to boiling.
(5) Cool (4) and filter.
(6) To each 100.0 cc. of (5) add 0.1%, 1.0%, 2.0%, 5.0% or 10.0% of one of (3). (Filtered extract solution.)
(7) Incubate at room temperature for one week to test sterility.

Sterilization: Sterilize for 20 minutes on each of 3 successive days in streaming steam.

Use: To study gum formation by *Bacillus radicicola.* Author reported that tolu, red clover blossoms, glycyrrhiza, kino, baptisia and senna favored production. Cathechu, physostigma, scoparius and kino in high concentrations were unsuitable.

Variants: The author added 1.0% maltose.

Reference: Buchanan (1909 pp. 72, 386).

1158. Buchanan's Basal Clover Infusion Solution

Constituents:

1. Water	4000.0 cc.
2. Clover leaves and stems (*Trifolium pratense*)	200.0 g.

Preparation:

(1) Extract 200.0 g. of the leaves and stems of clover (*Trifolium pratense*) with 4 liters of boiling tap water.
(2) Filter until clear.
(3) Add one of the added nutrients to (2).
(4) Heat to boiling.
(5) Cool and filter.
(6) Incubate for one week at room temperature to test sterility.

Sterilization: Sterilize for 20 minutes on each of 3 successive days in streaming steam.

Use: To study gum formation by *Bacillus radicicola,* and cultivation of *Bacillus radicicola* bacteroids. Author reported that clover extracts alone favored the production of gum. Peptone, and to a less degree, asparagin and sodium asparaginate inhibited the production of gum. Nitrates except in large amounts did not effect growth and gum production. Clover extract did not constitute a favorable medium for the development of the bacteroids, either unmodified or upon the addition of asparagin or peptone.

Added nutrients: The author added one of the following:

KH_2PO_4	0.2%
$MgSO_4$	0.1%
Ammonium phosphate	0.5%
Asparagin	1.0%
Sodium asparaginate	1.0%
Peptone	0.1, 0.5, 1.0, 2.0 or 5.0%
Sucrose	2.0%
KNO_3	0.005, 0.01, 0.05, 0.1, 0.2 0.5 or 1.0%
Glucose	2.0%

Reference: Buchanan (1909 pp. 62, 386, 391).

1159. Buchanan's Vetch Infusion Solution

Constituents:

1. Water	2000.0 cc.
2. Vetch stems and leaves	200.0 g.

Preparation:

(1) Boil 200.0 g. of dry vetch stems and leaves in 2 liters of water.
(2) Filter.
(3) Concentrate to $\frac{1}{6}$ its volume by heat.
(4) Add several times its volume of alcohol.
(5) Filter.
(6) Evaporate the filtrate to dryness.
(7) Wash the precipitate with absolute alcohol, several times and evaporate to dryness.
(8) Dissolve each of these extracts in 100.0 cc. of distilled water.
(9) Filter.
(10) The first of these is called alcoholic extract and the second the aqueous extract.
(11) Prepare 0.01, 0.1, 0.2 or 1.0% solutions of the aqueous extract in distilled water and 0.1, 0.5, 1.0, 2.0 or 5.0% solutions of the alcoholic extract in distilled water.
(12) Tube.
(13) Incubate for one week at room temperatures to test sterility.

Sterilization: Sterilize in streaming steam for 20 minutes on each of 3 successive days.

Use: To study gum formation by *Bacillus radicicola* and cultivation of *Bacillus* *radicicola* bacteroids. The author reported that the aqueous and alcoholic extracts in low dilutions tended to favor the production of gum. Both the alcoholic and aqueous extracts of the vetch served as suitable nutrients for growth of *B. radicicola* when in proper concentration. There was little bacteroid production.

Variants: The author specified the use of *V. Faba* stems or leaves.

Reference: Buchanan (1909 pp. 71, 386).

1160. Wilhelmi's Clover Flower Infusion Solution

Constituents:

1. Distilled water	1500.0 cc.
2. Clover flowers	
3. Glucose	75.0 g.

Preparation:

(1) Fill a 2 liter flask with clover flowers and add 1500.0 cc. distilled water and 75.0 g. glucose.
(2) Sterilize for 20 minutes in the autoclave at 120°C.
(3) Add sterile distilled water to restore to 1500.0 cc.
(4) Filter.
(5) Add 25.0% HCl until litmus paper is turned red (about 5.0 cc.).
(6) Allow to stand a day and again adjust (about 1 or 2 cc. acid will be required).
(7) A slight acidity will be reached after 4 or 5 days, then observe the reaction of the extract for 2 or 3 days.
(8) Add the required amount of HCl (best growth takes place when there is about 5.5 or 5.8% pure hydrochloric acid material present.

Sterilization: Sterilization is effected in step (3) above.

Use: Isolation of *Saccharomyces guttulatus* from intestines and stomach. Author reported that bacteria did not develop in this acid medium.

Reference: Wilhelmi (1898 p. 358).

1161. Reed and Cooley's Bean Pod Infusion Solution

Constituents:

1. Water.
2. Bean pods (*Phaseolus vulgaris*).

Preparation: (1) Cook green pods of *Phaseolus vulgaris* in water (amount not given).

Sterilization: Method not given.

Use: Cultivation of *Heterosporium variabile.*

Reference: Reed and Cooley (1911–12 p. 50).

1162. Mazé's Sucrose Bean Infusion Solution

Constituents:

1. Water	1000.0 cc.
2. Beans (white)	
3. Sucrose (2.0%)	20.0 g.
4. NaCl (1.0%)	10.0 g.

Preparation:

(1) Infuse white beans in water for 30 minutes at 100°C.
(2) Add 2.0% sucrose, 1.0% NaCl and a trace of sodium bicarbonate to (1). (Filtering of (1) not specified.)

Sterilization: Method not given.

Use: Cultivation of nitrogen fixing bacteria.

Variants:

(a) Tanner, Besson, and Dopter and Sacquépée prepared the medium as follows:
  (1) Macerate 50.0 to 60.0 g. haricot beans in a liter of water for several hours in the cold.
  (2) Boil for 30 minutes.
  (3) Pour on a coarse sieve.
  (4) Collect the liquid.
  (5) Add 1.0% NaCl, 2.0% sucrose and a pinch of sodium bicarbonate.
  (6) Boil and filter thru paper.
  (7) Tube.
  (8) Sterilize at 115°C.
(b) Harvey prepared the medium as follows:
  (1) Mince finely haricot beans.
  (2) Add 250.0 g. to 1000.0 cc. distilled water or clear tap water.
  (3) Heat the mixture 20 minutes at a temperature not exceeding 50°C.
  (4) Raise the temperature to boiling point.
  (5) Boil 10 minutes.
  (6) Add 1.0 cc. 1.0% sodium bicarbonate.
  (7) Add 10.0 g. sodium chloride.
  (8) Steam 45 minutes.
  (9) Bring the volume up to 1000.0 cc. by the addition of distilled water.
  (10) Filter while hot thru thick filter paper.

(11) Add 20.0 g. sucrose.
(12) Distribute into test tubes.
(13) Sterilize in the steamer or autoclave.

References: Mazé (1897 p. 45), Tanner (1919 p. 58), Besson (1920 p. 35), Dopter and Sacquépée (1921 p. 121), Harvey (1921–22 p. 119).

1163. de Rossi's Glucose Bean Infusion Solution

Constituents:

1. Water	1000.0 cc.
2. Beans 10.0%	100.0 g.
3. Glucose 2.0%	20.0 g.

Preparation:
(1) Prepare a bean infusion using 10.0% beans.
(2) Dissolve 2.0% glucose in (1).

Sterilization: Not specified.

Use: To study nitrogen fixation by bacteria from nodules of leguminous plants.

Reference: de Rossi (1909), (1910 p. 271).

1164. Kaufmann's Jequirity Infusion Solution

Constituents:

1. Water	1000.0 cc.
2. Jequirity seeds	100.0 g.

Preparation:
(1) Grind 100.0 g. Jequirity seeds in a mortar.
(2) The peeled or shelled seeds now weight about 80.0 g.
(3) Add to 1000.0 cc. of water.
(4) Boil in a steam sterilizer for about two hours.
(5) Cool and filter.
(6) Reaction is neutral or slightly alkaline.
(7) Distribute into test tubes.

Sterilization: Sterilize in the usual manner (method not given).

Use: General culture medium. Author reported that the medium supported the growth of *Bacillus pyocyaneus* and many other organisms.

Variants: The author aded 6.0% glycerol or 6.0% glycerol with 2.0% peptone.

Reference: Kaufmann (1891 pp. 65, 68).

1165. Tanner's Pea Flour Infusion Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Pea flour	100.0 g.
3. NaCl	100.0 g.

Preparation:
(1) Add 100.0 g. of pea flour and 100.0 g. NaCl to 1 liter distilled water.
(2) Mix and steam, stirring constantly for 30 minutes.
(3) Allow to settle.
(4) Filter.

Sterilization: Method not given.

Use: Peptone substitute. The author reported that this solution was especially designed to replace Witte's peptone. The infusion should, preferably, be made fresh each time it is used.

Reference: Tanner (1919 p. 57).

1166. Stutzer's Basal Legume Seed Infusion Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Pea seeds	10.0 g.
or	
clover seeds (*Trifol hybridum*)	10.0 g.
or	
bean seeds (*Vicia Faba*)	10.0 g.
or	
lupine seeds	10.0 g.

Preparation:
(1) Grind pea seeds, clover seeds (*Trifol hybridum*), bean seeds (*Vicia Faba*) or lupine seeds and pour 1 liter of water over each 10.0 g. of one of the ground seeds.
(2) Boil for 1 to 2 hours, adding the water lost by evaporation.
(3) Filter.
(4) Dilute or concentrate the filtrate as desired so that the ratio of pea seed to water be 100, 75, 50, 40, 30, 20, 10, 5, 4, 3, 2 or 1.0 g. per liter. Best results are obtained however using 10.0 g. peas per liter water as in (1).
(5) Dissolve one of the added nutrients in (4).

Sterilization: Not specified.

Use: The study the cultivation of *Pisum sativum, Vica Faba, Trifolium pratense* and *Trifolium incarnatum.* Author reported that generally the addition of carbohydrates and asparagin favored the growth of the bacteroids. Oil seemed to have little effect.

Added nutrients and variants:

(a) The author added one of the following:

Asparagin	2.0%
Glucose	1.0, 5.0 or 10.0%
Sucrose	1.0, 5.0 or 10.0%
starch	2.0%
flour	2.0%
inulin	2.0%
oil	small amount

(b) The author prepared an infusion from clover seeds in the following manner:

- (1) Heat 3.0 to 4.0 g. of clover seed in water on a water bath for several hours.
- (2) Pulverize the soft seeds in a rubbing dish.
- (3) Heat again for an hour.
- (4) Add distilled water until 1000.0 cc. are obtained (1.0 g. K_2HPO_4 may be added).
- (5) Distribute in 10.0 cc. lots.
- (6) Sterilize 3 times in the usual way. (Method not given.)

(c) The author added 0.1% K_2HPO_4 to an infusion prepared as indicated in the basic solution using 2.5 g. *Vicia Faba* per liter and added 0.0 or 1.0 g. K_2HPO_4.

(d) The author used the basic solution without any additions.

Reference: Stutzer (1900 pp. 898, 903, 908).

1167. Smith's Potato Infusion Solution

Constituents:

1. Water	1000.0 cc.
2. Potato	500.0 g.

Preparation:

- (1) Pass clean, pared potatoes rapidly thru a grating machine.
- (2) Throw immediately into double the weight of distilled water.
- (3) Place on a water bath and quickly raise the temperature to 55°C. Keep at this temperature for an hour, stirring frequently.
- (4) Filter.

Sterilization: Sterilize in the steamer.

Use: General culture medium. Author cultivated plant parasites.

Variants:

(a) Peklo cultivated plant actinomyces on a medium prepared as follows:

- (1) Prepare a pulp from raw potatoes.
- (2) Add a little distilled water (amount not given).
- (3) Allow to stand in the cold for 24 hours.
- (4) Press thru a towel.
- (5) Sterilize until there is no more coagulum formed (time not specified). (Filtration not specified.)

(b) Tanner prepared a similar medium as follows:

- (1) Wash and grind a few potatoes.
- (2) To 30.0 g. of (1) add a liter of distilled water.
- (3) Allow to stand in the ice box over night.
- (4) Filter.
- (5) Boil the filtrate.
- (6) Filter again if necessary.
- (7) Tube.
- (8) Sterilize (method not given).
- (9) If the reaction is too acid, adjust as usual.

(c) Besson prepared the medium as follows:

- (1) Clean and grate potatoes.
- (2) Soak 30.0 g. of (1) in 1000.0 cc. water for 4 hours.
- (3) Decant the liquid.
- (4) Boil.
- (5) Filter.
- (6) Reaction may be neutralized if desired.
- (7) Distribute as desired.
- (8) Sterilize in the autoclave or steamer.

(d) Dopter and Sacquépée prepared a similar medium as follows:

- (1) Grate potatoes.
- (2) Macerate 10.0 to 20.0 g. of (1) with 1 liter of water for two hours. (Temperature not specified.)
- (3) Strain thru a cloth.
- (4) Bring to a boil.
- (5) Filter thru paper.
- (6) Distribute.
- (7) Sterilize in the autoclave.

(e) Robertson and Davis studied the influence of vitamines on bacterial growth. They reported that the medium did not give continued growth of yeast. The medium was prepared as follows:

- (1) Grate potato fine and cover with sterile distilled water.

(2) Allow to stand at room temperature for 18 hours.
(3) Pass thru a Berkefeld filter.
(4) Adjustment of reaction not given.
(5) Add sterile (method of sterilization not given) physiological salt solution to (3).

(f) Park, Williams and Krumwiede prepared the medium as follows:
(1) Run white potatoes thru the grater or chopping machine.
(2) Mix 1.0 pound of (1) with water.
(3) Soak over night.
(4) Heat to boiling.
(5) Press thru cheese cloth.
(6) Add one egg per liter.
(7) Autoclave one hour.
(8) Filter thru cotton (very tedious).
(9) Store in flasks and sterilize in the autoclave for 30 minutes at 15 pounds pressure.

References: Smith (1905 p. 42), Peklo (1910 p. 551), Tanner (1919 p. 60), Besson (1920 p. 35), Dopter and Sacquépée (1921 p. 120), Robertson and Davis (1923 p. 154), Park, Williams and Krumwiede (1924 p. 122).

1168. Elsner's Hydrochinone Potato Infusion Solution

Constituents:
1. Potato water.
2. Hydrochinone.

Preparation:
(1) Preparation of potato water not given.
(2) Do not adjust the reaction.
(3) Add hydrochinone, amount not given.

Sterilization: Method not given. The medium turns brown during sterilization.

Use: Differentiation of coli and typhoid bacteria. Author reported that *B. coli* reduced the brown color. Typhoid bacteria gave no color change.

Reference: Elsner (1896 p. 28).

1169. Robertson and Davis' Potato Infusion Solution

Constituents:

1. Sterile distilled water	1000.0 cc.
2. Asparagin (Merck)	3.4 g.
3. $CaCl_2$	0.1 g.
4. Glucose	20.0 g.
5. $MgSO_4$	0.2 g.
6. K_2HPO_4	1.0 g.
7. NaCl	5.0 g.
8. Potato	

Preparation:
(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1 by boiling 3 minutes.
(2) Restore original volume with sterile distilled water.
(3) Adjust reaction to pH = 7.4.
(4) Tube.
(5) Grate potato fine and cover with sterile distilled water.
(6) Allow to stand at room temperatures for 18 hours.
(7) Pass thru a Berkefeld filter.
(8) Add various amounts of (7) to each tube of sterile (4).

Sterilization: Autoclave (3) at 20 pounds pressure for 30 minutes.

Use: To study influence of vitamines on bacterial growth. Authors reported that yeast grew very luxuriantly.

Reference: Robertson and Davis (1923 p. 154).

1170. Lubinski's Glycerol Potato Infusion Solution

Constituents:

1. Water	1500.0 cc.
2. Potato	1000.0 g.
3. Glycerol (4.0%)	60.0 g.

Preparation:
(1) Wash potatoes clean and cut them in small pieces.
(2) Boil (1) for 3 or 4 hours under a free flame, or in a steamer, in 1500.0 cc. of water.
(3) Filter.
(4) Add 4.0% glycerol to the filtrate.
(5) Neutralize (may be used without neutralization).

Sterilization: Method not specified.

Use: Cultivation of tubercle bacilli.

Variants:

(a) Harvey prepared the medium as follows:
(1) Grate finely washed peeled potatoes.
(2) Add 1000.0 to 2000.0 cc. water.
(3) Heat the mixture 20 minutes to a temperature not exceeding 50°C.
(4) Raise to boiling temperature.
(5) Boil 10 minutes.

(6) Pour the mixture on a clean, thick cloth.
(7) Collect the fluid which drains thru the cloth together with that obtained by squeezing the cloth.
(8) Filter the fluid collected thru thick filter paper.
(9) Add the filtrate to an equal quantity of distilled water.
(10) Steam 60 minutes.
(11) Add glycerol to 4 per cent.
(12) Mix well.
(13) Filter.
(14) Distribute into test tubes.
(15) Sterilize in the autoclave or steamer.

(b) Harvey prepared a medium similar to the above as follows:
(1) Grate finely washed, peeled potatoes.
(2) Add 2 parts 4 per cent glycerol to 1 part potato gratings.
(3) Boil.
(4) Filter.
(5) Use the filtrate as potato extract.

References: Lubinski (1895 p. 126), Harvey (1921–22 pp. 118, 119).

1171. Berthelot's Vegetable Infusion Solution

Constituents:

1. Water	4000.0 cc.
2. Potato	300.0 g.
3. Carrots	150.0 g.
4. Turnips	150.0 g.

Preparation:
(1) Peel the potatoes. They may be left whole or cut in two.
(2) Wash the carrots and turnips.
(3) Mix (1) and (2) in 4 liters of cold water.
(4) Bring to boil and boil for 4 hours in order to reduce the volume of water one-fourth.
(5) Filter thru a fine towel.
(6) Bring the filtrate to 3 liters volume either by further boiling or adding distilled water.
(7) Add 10.0% soda solution to neutralize boiling (6). The reaction may be adjusted to a slight alkalinity to litmus.
(8) Heat in the autoclave at 120° for 30 minutes.
(9) Allow to stand for 24 hours in the cold or better on ice.
(10) Filter thru paper.
(11) Distribute as desired.

Sterilization: Sterilize at 115° for 20 minutes.

Use: General culture medium for saprophytes and pathogenic forms.

Variants: Tanner gave the following method of preparation:
(1) Peel the potatoes.
(2) Wash the carrots and turnips.
(3) Cut (1) and (2) in small pieces and place in cold water.
(4) Boil 4 hours.
(5) Strain.
(6) Make weakly alkaline.
(7) Heat in autoclave at 120°C.
(8) Allow to stand in a refrigerator over night.
(9) Filter thru paper.
(10) Final sterilization not given.

References: Berthelot (1917 p. 131), Tanner (1919 p. 46).

1172. Migula's Potato Juice

Constituents:
1. Potato.

Preparation:
(1) Pass peeled potatoes thru a sieve, or grind real fine.
(2) Press the juice thru a linen towel.
(3) Distribute in tubes.

Sterilization: Boil for 20 minutes on each of 3 successive days in a steamer.

Use: General culture medium.

Reference: Migula (1901 p. 20).

1173. Winogradsky's Gypsum Root Solution

Constituents:

1. Water	3000.0 to 5000.0 cc.
2. Gypsum	
3. "Butomus-Rhizonus"	

Preparation:
(1) Cut a "Butomus-Rhizomus," freshly removed from the ground, into several pieces.
(2) Place the pieces containing mud or slime into a deep jar, containing 3.0 to 5.0 liters of water.
(3) Add a few grams of gypsum.

Sterilization: Not specified.

Use: Enrichment of sulphur bacteria. Author reported that H_2S was formed after 5 to 7 days. The slime at the bottom became black. The liquid became opalescent and surface 3 to 6 weeks a membrane of sulfur formed on the surface.

Reference: Winogradsky (1888 p. 11).

1174. Tanner's Carrot Infusion Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Carrots	30.0 g.

Preparation:

(1) Wash and grind a few carrots.
(2) To 30.0 g. of (1) add a liter of distilled water.
(3) Allow to stand in the ice box over night.
(4) Filter.
(5) Boil the filtrate.
(6) Filter again if necessary.
(7) Tube.
(8) If the reaction is too acid, adjust as usual.

Sterilization: Method not given.

Use: General culture medium.

Variants:

(a) Besson soaked the carrots for 4 hours instead of over night, as in step (3) above.
(b) Dopter and Sacquépée prepared the medium as follows:
  (1) Grate carrots.
  (2) Macerate 10.0 to 20.0 g. of (1) with 1 liter of water for 2 hours. (Temperature not specified.)
  (3) Strain thru a cloth.
  (4) Bring to a boil.
  (5) Filter thru paper.
  (6) Distribute.
  (7) Sterilize in the autoclave.
(c) Robertson and Davis studied the influence of vitamines on bacterial growth. They prepared a medium as follows:
  (1) Grate carrots fine, and cover with sterile distilled water.
  (2) Let stand at room temperatures for 18 hours.
  (3) Pass thru a Berkefeld filter.
  (4) Adjustment of reaction not given.
  (5) Add to (3) sterile (method of sterilization not given) physiological salt solution. Amounts not specified.

  They reported that the medium did not give continued growth of yeast.
(d) Draper gave the following method of preparation. The medium was used for the cultivation of *Oidium albicans*.
  (1) Chop raw carrots into fine particles and allow to extract in sterile distilled water at about 10°C. for 48 hours.
  (2) Filter.
  (3) Tube.
  (4) Autoclave for 15 minutes at 15 pounds pressure.

References: Tanner (1919 p. 60), Besson (1920 p. 35), Dopter and Sacquépée (1921 p. 120), Robertson and Davis (1923 p. 154), Draper (1924 p. 635).

1175. Robertson and Davis' Carrot Infusion Solution

Medium identical with medium 1169 but carrot used instead of potato.

1176. Migula's Hay Infusion Solution

Constituents:

1. Water	3000.0 cc.
2. Hay	100.0 g.

Preparation:

(1) Pour 3 liters of water over 100.0 g. of hay and allow to stand for 24 hours in a warm place.
(2) Press thru linen.
(3) Neutralize and make slightly alkaline to litmus.
(4) Boil for one hour.
(5) Cool to 40°C. and add the whites of 3 eggs.
(6) Boil and filter.
(7) Distribute in 100.0 cc. lots in flasks.

Sterilization: Steam on 3 successive days for one hour each day.

Use: General culture medium.

Variants: The following methods of preparation have been suggested by various investigators:

(a) Heinemann.
  (1) Macerate 10.0 g. of chopped hay in 1000.0 g. water in the water bath for 3 hours.
  (2) Filter.
  (3) Sterilize in the autoclave for 10 minutes at 120°C.
(b) Linde cultivated Cladothrix in a light yellow completely transparent hay infusion, or in a coffee brown hardly transparent medium. The

media were employed without any additions, or made slightly alkaline by the addition of Na_2CO_3.

(c) Tausz and Peter.

(1) Wash grass that has been piled up for a few days and only slightly decomposed, with a little water to remove the dirt.
(2) Boil with a little water.
(3) Cut the grass into small pieces and boil for 20 minutes with 3.5 liters of water.
(4) Filter. The filtrate is dark brown.
(5) Neutralize by the addition of several drops of KOH.
(6) Sterilize by steaming on each of 3 successive days for 30 minutes.

They cultivated *Bacterium aliphaticum, Bacterium aliphaticum liquefaciens*, paraffin bacteria.

(d) Tanner, Besson, and Dopter and Sacquépée.

(1) Macerate 15.0 to 20.0 g. of finely chopped hay in 1000.0 g. of water for one or two hours.
(2) Boil a few minutes.
(3) Filter.
(4) Tube.
(5) Sterilize at 115°C.
(6) The infusion may be neutralized if the reaction is a little acid.

Besson, and Dopter and Sacquépée treated straw in exactly the same manner.

(e) Harvey.

(1) Prepare dried chopped hay 10; distilled water 1000, in a well-stoppered bottle.
(2) Heat to 70°C.
(3) Close tightly with stopper.
(4) Heat 3 hours at 60°C. on a water bath.
(5) Steam 60 minutes.
(6) Filter thru thick filter paper.
(7) Distribute into test tubes.
(8) Sterilize in the autoclave or steamer.

(f) Harvey.

(1) Mince finely hay or straw.
(2) Add 15 to 20.0 g. to 1000.0 cc. water.
(3) Heat the mixture at a temperature not exceeding 50°C.
(4) Raise the temperature rapidly to boiling point.
(5) Boil 10 minutes.
(6) Adjust reaction.
(7) Filter.
(8) Distribute into flasks or test tubes.
(9) Sterilize in the autoclave or steamer.

References: Migula (1901 p. 20), Heinemann (1905 p. 129), Linde (1913 p. 386), Tausz and Peter (1919 p. 509), Tanner (1919 p. 57), Besson (1920 p. 35), Dopter and Sacquépée (1921 p. 120), Harvey (1921–22 pp. 120, 121).

1177. Winogradsky's Ferric Hydroxide Hay Infusion Solution (Molisch)

Constituents:

1. Water.
2. Hay.
3. Ferric hydroxide.

Preparation:

(1) Place a handful of macerated hay that has been well extracted with water in a glass cylinder 50 cm. high.
(2) Sprinkle some freshly precipitated ferric hydroxide on the hay.
(3) Fill the cylinder with well water.

Sterilization: Not specified.

Use: Cultivation of iron bacteria, *Chlamydothrix* (*Leptothrix*) *ochracea.*

Reference: Molisch (1910 p. 32).

1178. Jensen's Nitrate Straw Solution

Constituents:

1. Water	100.0 cc.
2. Straw	5.0 g.
3. $NaNO_3$	1.0 g.

Preparation: (1) Mix 2 and 3 with 1.

Sterilization: Not specified.

Use: To study denitrification by straw forms. The author reported that glycerol aided in the reduction of nitrates.

Variants: The author added 2.0% glycerol.

Reference: Jensen (1897 p. 691).

1179. Wolbach and Binger's Glucose Hay Infusion Solution

Constituents:

1. Water	1000.0 cc.
2. Hay	120.0 g.
3. Glucose	10.0 g.

Preparation:

(1) Cut 120.0 g. hay fine and soak in 1 liter of water for ½ hour. Final reac-

tion being about 0.2% acid to phenolphthalein.
(2) Decant the water and add 1.0% glucose.
(3) Adjust the reaction to neutral to phenolphthalein.
(4) Tube after sterilization.

Sterilization: Sterilize by filtration thru Chamberland F filter.

Use: Cultivation of *Spirocheta elusa* (free living). Author reported that the medium was clouded by growth in about 48 hours. Medium not so satisfactory if sterilized by repeated steaming or in the autoclave.

Reference: Wolbach and Binger (1914 p. 9).

1180. Sherman's Soil Hay Infusion Solution

Constituents:

1. Distilled water	900.0 cc.
2. Soil	100.0 g.
3. Hay infusion (1.0%)	100.0 cc.
4. $CaCO_3$	small excess

Preparation:
(1) Boil 1 and 2.
(2) Filter clear.
(3) Add 3.
(4) Add 4 in small excess.

Sterilization: Method not given.

Use: Cultivation of protozoa, flagellates, ciliates and amoebae.

Reference: Sherman (1916 p. 42).

1181. Smith's Cabbage Infusion Solution

Constituents:
1. Distilled water.
2. Cabbage leaves.

Preparation: (1) Boil a few cabbage leaves in distilled water.

Sterilization: Method not given.

Use: Cultivation of *Pseudomonas campestris* (Pammel). Author reported that organisms grew very well in the medium producing a heavy clouding and a copious yellow precipitate. *B. cloacae* gave gas from this medium. In litmus medium, the culture continued to be red for several days. Then the litmus lost its color except in the very top layer where it came in contact with air.

Variants: The author added sufficient Trommsdorf's litmus to color the solution a pale red. Re-sterilize.

Reference: Smith (1897 p. 479).

1182. Conrad's Glucose Cabbage Infusion Solution

Constituents:

1. Water	200.0 cc.
2. Cabbage	100.0 g.
3. Glucose	3.0%
4. NaCl	0.5%

Preparation:
(1) Prepare an extract of cabbage (Weisskraut abkochung) in the ratio of 1:2. Method not given.
(2) Add 3.0% glucose and 0.5% NaCl to (1).
(3) Place in a large flask.

Sterilization: Sterilize on 3 successive days in the steamer.

Use: To study fermentation by sauerkraut bacteria (*Bacterium brassicae acidae*).

Reference: Conrad (1897 p. 65).

1183. Will's Cabbage Juice

Constituents:
1. Cabbage juice.

Preparation: (1) Method of obtaining cabbage juice not given.

Sterilization: Method not given.

Use: Cultivation of mycoderma. Fred and Peterson studied the effect of different NaCl concentrations on the growth of lactic acid producing bacteria. They reported that the higher the salt concentration the less acid produced, 4.0% was the critical point. Yeast grew well at 4.0% or higher NaCl concentrations.

Variants: Fred and Peterson added 0.0, 1.0, 2.0, 4.0, 5.0 or 6.0% NaCl to cabbage juice.

References: Will (1910 p. 3), Fred and Peterson (1922 p. 266).

1184. Reed and Cooley's Basal Spinach Infusion Solution

Constituents:

1. Water	500.0 cc.
2. Spinach	100.0 g.

Preparation:
(1) Steep 100.0 g. of young spinach leaves in 500.0 cc. water.
(2) Filter.
(3) Neutralize.
(4) Add H_2SO_4 to make slightly acid to litmus.
(5) Dissolve 1.0% of one of the added nutrients in (4).

Sterilization: Not specified.

Use: Cultivation of *Heterosporium variabile*.

Added nutrients: The author added 1.0% NH_4NO_3 or 1.0% peptone.

Reference: Reed and Cooley (1911–12 p. 50).

1185. Sucksdorff's Tea Infusion

Constituents:

1.	Water	1000.0 cc.
2.	Tea	10.0 g.

Preparation:

(1) Place 10.0 g. tea in a liter of boiling water.

(2) After 5 minutes heating filter half the tea into a clean flask. Continue heating the other half for 5 minutes and then filter into a clean flask.

(3) Distribute into clean non-sterile beakers and place in an open window.

Sterilization: Do not sterilize.

Use: To study the type of organisms that will grow in tea. The author reported that schizomycetes grew better in tea than coffee. Molds, however, grow better in coffee (1192) than tea.

Reference: Sucksdorff (1886 p. 368).

1186. Bushnell's Asparagus Solution

Constituents:

1.	Tap water	300.0 cc.
2.	Asparagus	100.0 g.

Preparation: (1) Suspend asparagus in water (method not given).

Sterilization: Autoclave at 20 pounds for 30 minutes.

Use: To study changes (chemical) produced by anaerobes, *B. sporogenes* group.

Reference: Bushnell (1922 p. 381).

1187. Tausz and Peter's Leaf Infusion Solution

Constituents:

1.	Water	3500.0 cc.
2.	Leaves, decomposed	70.0 g.

Preparation:

(1) Boil 70.0 g. of decomposed leaves in 3.5 liters of water for about 40 minutes.

(2) Filter.

(3) Neutralize by the addition of several drops of KOH.

(4) Distribute in flasks and plug.

Sterilization: Sterilize by steaming for 30 minutes on each of 3 successive days.

Use: Cultivation of *Bacterium aliphaticum*, *Bacterium aliphaticum liquefaciens*, paraffin bacteria.

Variants: The author gave the following method of preparation.

(1) Pour boiling water over 150.0 g. fresh green maple leaves and then cut into small pieces.

(2) Boil with 3.5 liters of water for 30 minutes.

(3) Filter or clarify by allowing the material to stand. The filtrate is green. Filtration proceeds slowly.

(4) Neutralize by the addition of KOH until phenolphthalein is colored slightly red.

(5) Flask and plug.

(6) Sterilize by steaming for 30 minutes on each of 3 successive days.

Reference: Tausz and Peter (1919 p. 49).

1188. Müller-Thurgau's Grape Vine Infusion Solution

Constituents:

1.	Water	1000.0 cc.
2.	Grape vine (stems)	100.0 g.
3.	Sucrose	50.0 g.

Preparation:

(1) Prepare an infusion of 100.0 g. of grape vine (stems) in 1000.0 cc. of water, method not given.

(2) Add 5.0% sucrose to (1).

(3) Dissolve 1.0% $Ca_3(PO_4)_2$ and 0.5% $MgSO_4$ in (2).

Sterilization: Method not given.

Use: Cultivation of red burning fungus of grape vines. *Pseudopeziza tracheiphila* Author reported that fungus grew luxuriant at first; then began to turn brown and to show different degeneration products.

Variants: The author omitted the $Ca_3(PO_4)_2$ and $MgSO_4$ and added 5.0% bouillon.

Reference: Müller-Thurgau (1903 p. 82).

1189. Malenkovic's Wood Infusion Solution

Constituents:

1.	Water	1000.0 cc.
2.	KNO_3	1.5 g.
3.	K_2HPO_4	1.5 g.
4.	$NH_4H_2PO_4$	1.0 g.

5. $MgSO_4$	0.5 g.
6. Wood	

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Extract 1000.0 cc. wood with (1). (Method not given.)

Sterilization: Not specified.

Use: Cultivation of *Coniophora cerebella* (*Corticium putaneum*). Author reported good growth.

Reference: Malenkovic (1906 p. 409).

1190. Olitsky's Tomato Infusion

Constituents:

1. Distilled water	250.0 cc.
2. Tomato plants	80.0 g.

Preparation:
(1) Select 80.0 g. of fresh young stems, leaves and shoots of a healthy tomato plant.
(2) Cut in small pieces and grind by hand to a soft pulp in a large mortar.
(3) Thoroughly mix with 250.0 cc. of sterile distilled water, having a pH between 5.3 and 5.4.
(4) Centrifuge at a high speed for one hour.
(5) Medium discarded if not crystal clear, or reaction does not lie within 5.3 and 6.0 on the Sörensen scale.

Sterilization: Pass the supernatant fluid through two sterile Berkefeld filters size N. Incubate at 7 days at 28–30°C.

Use: Cultivation of the active agent of mosaic of tobacco and tomato.

Reference: Olitsky (1925 p. 131).

1191. Schardinger's Prune Solution

Constituents:

1. Water	200.0 cc.
2. Prunes	500.0 g.
3. $CaCO_3$	10.0 g.

Preparation: (1) Sterilize 200.0 cc. water, 500.0 g. of pitted prunes and 10.0 g. $CaCO_3$.

Sterilization: Given in step (1) above.

Use: Cultivation of *Bacillus macerans* and used as a general culture medium.

Variants: Tanner prepared a similar medium as follows:
(1) Macerate 50.0 g. of prunes in 1 liter of distilled water.
(2) Allow to stand in the ice chest until next morning.
(3) Filter.
(4) Tube.
(5) Sterilize in the autoclave or steamer.

References: Schardinger (1907 p. 161), Tanner (1919 p. 57).

1192. Sucksdorff's Coffee Infusion

Constituents:

1. Water	1000.0 cc.
2. Coffee	50.0 g.

Preparation:
(1) Pour 1 liter of boiling water over 50.0 g. coffee.
(2) Boil for 2 to 3 minutes.
(3) Filter thru a double filter into a clean flask.
(4) Distribute into clean non-sterile beakers and place in an open window.

Sterilization: Do not sterilize.

Use: To study the type of organisms that develop in coffee. Author reported that molds grew well in the coffee, while schizomycetes grew better in tea (1185).

Reference: Sucksdorff (1886 p. 368).

1193. Tanner's Fruit Infusion Solution

Constituents:

1. Water	1000.0 cc.
2. Fruit (dried)	50.0 to 100.0 g.

Preparation:
(1) Macerate 50.0 to 100.0 g. of dried fruit in a liter of water for several hours.
(2) Stew (1) in the water (time not given).
(3) Pass thru a coarse sieve.
(4) Boil.
(5) Filter.
(6) Tube.

Sterilization: Sterilize at 115°C.

Use: Cultivation of molds.

Variants: Harvey used bananas, prunes or raisins and proceeded as follows:
(1) Mince finely bananas, prunes or raisins.
(2) Add 75.0 g. to 1000.0 cc. of water.
(3) Heat the mixture 20 minutes at a temperature not exceeding 50°C.
(4) Raise the temperature to boiling point.
(5) Boil 10 minutes.
(6) Strain the fluid thru a thick, clean cloth and squeeze out the residue of the fluid.

(7) Adjust the reaction.
(8) Filter thru well-wetted, thick filter paper.
(9) Distribute into flasks or test tubes.

References: Tanner (1919 p. 59), Besson (1920 p. 36), Harvey (1921–22 p. 119).

1194. Turner's Quince Seed Infusion Solution

Constituents:
1. Distilled water.
2. Quince seed.

Preparation:
(1) Boil quince seeds in distilled water.
(2) Pass the thick glutinous mass thru a sieve.
(3) Dilute with distilled water to obtain the desired consistency.

Sterilization: Not specified.

Use: Cultivation of Euglena.

Reference: Turner (1917 p. 239).

1195. Müller-Thurgau and Osterwalder's Grape Juice

Constituents:
1. Grape juice.

Preparation: (1) Preparation of grape juice not given.

Sterilization: Not specified.

Use: To study fermentation by *Bacterium mannitopeum*, *Bacterium intermedium*, *Bact. Gayoni*, Mannite bacteria from wine, *Bacterium gracile*, *Micrococcus acidovorax*, *Micrococcus variococcus*.

Reference: Müller-Thurgau and Osterwalder (1918 p. 17).

1196. Schukow's Grape Must Solution

Constituents:

1. Water	300.0 cc.
2. Grape must	100.0 cc.
3. Malic acid (9.0 to 10.0%)	36.0 to 40.0 g.

Preparation:
(1) Dilute one part grape juice with 3 parts water.
(2) Add 9.0 to 10.0% of malic acid to (1).
(3) Distribute into fermentation flasks (sealed with H_2SO_4).

Sterilization: Sterilize in a steamer.

Use: To study the utilization of acid by yeast.

Variants: The author gave the following variants:
(a) Used tartaric acid instead of malic.
(b) Used 0.3% tartaric acid and 0.3% malic acid instead of malic acid alone.
(c) Heinemann prepared a similar medium as follows:
  (1) Dilute wine must with 4 times its weight of water.
  (2) Dissolve 0.5% ammonium tartrate in (1).
  (3) Macerate in the water bath for 1 hour.
  (4) Filter.
  (5) Sterilize in the Arnold for 3 consecutive days.

References: Schukow (1896 pp. 608, 610), Heinemann (1905 p. 129).

1197. Perold's Wine Medium

Constituents:
1. Spätburger wine.

Preparation: (1) Neutralize completely fermented Spätburger wine of an acidity of 9.15 g. wine acid per liter, to 5.0% acetic acid by the addition of 21.28% KOH.

Sterilization: Method not given. After sterilization the medium had the following composition:
Total acid 5.0% acetic acid.
Alcohol content 8.5% by volume.
Volatile acid 0.39% acetic acid.

Use: Cultivation of bacteria found in wine.

Variants:
(a) Müller-Thurgau and Osterwalder used red or white wine. Wine may or may not be sterilized. Used to study fermentation of *Bacterium mannitopoeum Bacterium intermedium*, *Bact. Gayoni* mannitol bacteria from wine.
(b) Tanner and Besson neutralized the wine before sterilization.

References: Perold (1909 p. 18), Müller-Thurgau and Osterwalder (1918 p. 19), Tanner (1919 p. 57), Besson (1920 p. 36).

1198. Bierberg's Currant Must Solution

Constituents:

1. Water	4000.0 cc.
2. Currant must	1000.0 cc.
3. NH_4Cl	20.0 or 40.0 g.

Preparation:
(1) Add 1 liter of currant must (800 Oechsle) to 4 liters of water.

(2) Dissolve 20.0 or 40.0 g. of NH_4Cl in (1).

Sterilization: Not specified.

Use: To study fermentation by yeast. Author reported that the addition of ammonium salts seemed to hasten the CO_2 production. The higher amount of salts gave a little more CO_2 at first. Over a long period of time there was little difference in the amount of CO_2 formed.

Variants: The author gave the following variants:

(a) Used 20.0 or 40.0 g. of ammonium phosphate instead of NH_4Cl.
(b) Omitted the NH_4Cl.

Reference: Bierberg (1909 p. 18).

1199. Müller's Currant Must Solution

Constituents:

1. Water	1200.0 cc.
2. Currant juice	400.0 cc.
3. Sucrose	200.0 g.

Preparation:

(1) Add 400.0 cc. of currant juice to 1200.0 cc. water.
(2) Dissolve 200.0 g. sucrose in (1).

Sterilization: Not specified.

Use: To study fermentation by yeast. The author reported that after three and a half months wine was formed. The type of wine formed varied little whether the juice was taken from normal or diseased currants.

Reference: Müller (1909 p. 157).

1200. Ottolenghi's Melon Juice Solution

Constituents:

1. Melon juice	1000.0 cc.
2. Na_2CO_3 (3.0% soln.) (10.0%).	100.0 cc.

Preparation:

(1) Method of obtaining melon juice not specified. Maturity of melons not specified.
(2) Neutralize the juice with 3.0% Na_2CO_3 and add 10.0%.
(3) Flask.
(4) Sterilize in the autoclave.
(5) Filter.

Sterilization: Sterilize in the autoclave following filtration.

Use: Enrichment media for cholera vibrio. The author reported that in melon juice the cholera vibrio developed much like in peptone water. After a short time, however, the alkali was neutralized and development stopped. Melon juice was superior to cucumber juice.

Variants: The author substituted cucumber juice for melon.

Reference: Ottolenghi (1911 p. 370).

1201. Bierberg's Cherry Must Solution

Constituents:

1. Water	2000.0 cc.
2. Cherry must	1000.0 cc.
3. Sucrose	

Preparation:

(1) The cherry must primarily should have a must weight of 66° Oechsle and 22.9 Prs. M. acid.
(2) Dilute 1 liter of (1) with 2 liters of water.
(3) Add sucrose to (2) until the must weight is 140° Oechsle.

Sterilization: Not specified.

Use: To study fermentation by yeast. The author reported that the addition of ammonium salts seemed to hasten the CO_2 production. The higher amount of salts gave a little more CO_2 at first. Over a long period of time there was little difference in the amount of CO_2 formed.

Variants: The author gave the following variants:

(a) The addition of 20.0 g. or 40.0 g. of ammonium phosphate.
(b) The addition of 20.0 or 40.0 g. of NH_4Cl.

Reference: Bierberg (1909 p. 18).

1202. Müller-Thurgau and Osterwalder's Pear Juice Solution

Constituents:

1. Pear juice.

Preparation: (1) Preparation of pear juice not given.

Sterilization: Not given.

Use: To study fermentation by *Bacterium mannitopoeum, Bacterium, intermedium, Bact. Gayoni*, mannitol from wine.

Variants: The authors specified that malic acid or alcohol might be added to the pear juice.

Reference: Müller-Thurgau and Osterwalder (1918 p. 48).

1203. Giltner's Fermented Cider

Constituents:

1. Cider.

Preparation:

(1) Inoculate unfermented cider with *Sacch. ellipsoideus*, and allow to proceed until the evolution of gas ceases.
(2) Filter.
(3) Tube or flask as desired.

Sterilization: Pasteurize. Time and temperature not specified.

Use: Cultivation of acetic acid bacteria.

Reference: Giltner (1921 p. 370).

1204. Zikes' Nitrate Fruit Must Solution

Constituents:

1. Prune must	
2. $NaNO_3$	1.0, 5.0 or 10.0%

Preparation:

(1) Preparation of prune must not given.
(2) Add 0.0, 1.0, 5.0 or 10.0% of $NaNO_3$ to (1).

Sterilization: Not specified.

Use: Volutin production by yeast. *Oidium lactis.* Author reported that the presence of nitrate tended to decrease the number of yeast.

Variants: The author used raisin must instead of prune must.

Reference: Zikes (1922 p. 33).

1205. Schukow's Fruit Must Solution

Constituents:

1. Grape must.

Preparation:

(1) Prepare grape must of 16.8° Balling.
(2) Distribute into fermentation flasks (sealed with H_2SO_4).

Sterilization: Sterilize in a steamer.

Use: To study utilization of acid by yeast. Author reported that after 85 days 0.112 g. of acid were utilized from grape must, 0.25 g. acid from currant must, and 0.05 g. acid from apple must. Acid determined by titration using litmus as an indicator. Used by Chrzaszcz for the cultivation of amöba.

Variants: Author used the following instead of grape must:

(a) Currant must of 14.4° Balling.
(b) Currant must of 14.4° Balling diluted with an equal volume of water.
(c) Apple must of 20.0° Balling.
(d) Chrzaszcz cultivated *Physarum leucophaeum* (amöba) on grape must or pear must (*Monilia fructigenes*).

References: Schukow (1896 p. 609), Chrzaszcz (1902 p. 433).

1206. Bachmann's Mince Meat Medium

Constituents:

1. Water	900.0 cc.
2. Chopped apple	1
3. Raisins	1 cup
4. Butter	¼ lb.
5. Vinegar	1 tbsp.
6. Sugar	1 cup
7. Steak, hamburger	½ lb.
8. Cinnamon	1 tsp.
9. Cloves	1 tsp.
10. Nutmeg	1 tsp.
11. Mace	1 tsp.
12. NaCl	1 tsp.

Preparation:

(1) Mix 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 in enough water to make 900.0 cc.
(2) Cook in the steamer for 30 minutes.
(3) Distribute in 6 inch test tubes and fill them half full.

Sterilization: Sterilize in the autoclave.

Use: To study effect of spices on growth of *Clostridium botulinum.* Author reported that *Clostridium botulinum* grew readily in the medium.

Variants: The author used beef suet instead of butter.

Reference: Bachmann (1923 p. 237).

1207. Harvey's Wheat Flour Solution

Constituents:

1. Water	1000.0 cc.
2. Glucose	15.0 g.
3. KNO_3	1.0 g.
4. $MgSO_4$	0.5 g.
5. Wheat flour	150.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1. (Further preparation not given.)

Sterilization: Not specified.

Use: General culture medium.

Reference: Harvey (1921–22 p. 119).

1208. Omeliansky's Flax Stem Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium phosphate	1.0 g.
3. Potassium phosphate	1.0 g.

4. $MgSO_4$ .... 0.5 g.
5. NaCl .... trace
6. Flax stems
7. Chalk

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Cut the flax stems in pieces about 7.0 cm. long and tie in little bunches.
(3) Place these bunches in a long necked flask holding about 300.0 cc.
(4) Shake a little chalk in the bottom of the flask.
(5) Fill the flask with (1).

Sterilization: Not specified.

Use: To study changes produced in flax stems by pectin and cellulose fermenters.

Reference: Omeliansky (1904 p. 35).

1209. Speakman and Phillips' Maize Mash Solution

Constituents:

1. Water .... 3000.0 cc.
2. Maize .... 10.0 g.

Preparation: (1) Prepare a 3.0% maize mash (method not given).

Sterilization: Sterilize in the usual manner (details not specified).

Use: Cultivation of *B. granulobacter-pectinovorum, B. volutans, B. mesentericus.*

Reference: Speakman and Phillips (1924 p. 186).

1210. Lafar's Beer Solution

Constituents:

1. Water.
2. Lager beer.
3. Alcohol.

Preparation:

(1) Boil lager beer to remove the alcohol and make up the loss by the addition of water.
(2) Distribute in 125.0 cc. lots in Erlenmeyer flasks, plug the flasks with cotton, and place a paper cap on each flask.
(3) Before use, add 6.0 cc. alcohol to each flask of sterile (2).

Sterilization: Sterilize on 3 successive days in streaming steam for 25 minutes.

Use: To study lactic acid production by *Bacterium Pasteurianum* and *B. aceti H.*

Variants: Lafar prepared the medium as follows:

(a) Heat 50.0 cc. of Lager beer in Erlenmeyer flasks on 3 successive days for 20 minutes. Add 2.0 cc. alcohol before inoculation.
(b) Heat 100.0 cc. of light Lager beer in Erlenmeyer flasks on 3 successive days for 30 minutes. Add 5.0 cc. of alcohol before inoculation.
(c) Dilute 50.0 cc. light Lager beer that has been boiled to remove the alcohol with 75.0 cc. water. Heat 3 times for 25 minutes each and add 5.0 cc. alcohol to each flask.
(d) Heat dark beer in a steamer 3 times for 25 minutes each.
(e) Heat dark beer in 125.0 cc. lots in Erlenmeyer flasks 3 times for 25 minutes each. Add 5.0 cc. alcohol.
(f) Dilute 50.0 cc. of dark beer with 25.0 cc. water and heat 3 times for 20 minutes each. Add 2.0 cc. of alcohol to each flask.
(g) Janke prepared a similar medium as follows:
    (1) Distribute Austrian double malt beer in 10.0 cc. lots in Freudenreich flasks.
    (2) Sterilize on each of 3 successive days for 20 minutes in the steamer.
    (3) The alcohol content remains over 2.2% by volume.
(h) Janke prepared a Lager beer medium as follows:
    (1) Evaporate Lager beer to ½ its original volume by steaming.
    (2) Add water to its original volume.
    (3) Distribute in 10.0 cc. lots in Freudenreich flasks.
    (4) Sterilize on 3 successive days in the steamer.
    (5) Add 3.0% by volume of absolute alcohol.

References: Lafar (1895 pp. 132, 140), Janke (1916 p. 5).

1211. Waterman's Sucrose Beer Solution

Constituents:

1. Beer .... 1000.0 cc.
2. Sucrose .... 40.0 g.

Preparation: (1) Dissolve 40.0 g. of sucrose in beer.

Sterilization: Method not specified.

Use: Cultivation of vinegar bacteria, mycoderma, *B. Pasteurianum, B. rancens,* *B. aceti*, *Acetobacter melanogenum*, *B. xylinum*.

Reference: Waterman (1913 p. 452).

1212. Würtz's Malt Infusion Solution

Constituents:

1. Water	1000.0 cc.
2. Malt	100.0 g.

Preparation:

(1) Grind 100.0 g. of malt.
(2) Add (1) to 1000.0 cc. of water.
(3) Heat for one hour between 55–58°C. (not above 58°).
(4) Boil.
(5) Filter.

Sterilization: Sterilize at 115° for 15 minutes.

Use: Cultivation of yeast, molds and trichophyta.

Variants:

(a) Thoinot and Masselin prepared a similar medium from malt dust as follows:
  (1) Wash 100.0 g. of malt dust with water.
  (2) Boil in a liter of water.
  (3) Filter.
  (4) Distribute as desired.
  (5) Sterilize at 115° for 15 minutes.
(b) Tanner prepared a similar medium as follows:
  (1) Place 250.0 g. of malt into a flask.
  (2) Add 1000.0 cc. of distilled water heated to 70°C. to (1), and close with a rubber stopper.
  (3) Place in a water bath at 60°C. for 1 hour.
  (4) Strain thru cheese cloth into clean flasks.
  (5) Heat in an Arnold for 30 minutes.
  (6) Filter thru paper.
  (7) Tube in 10.0 cc. quantities.
  (8) Sterilize in an Arnold.
(c) Dopter and Sacquépée prepared a medium in the same manner as variant (a) above, but used only 50.0 g. of malt dust instead of 100.0 g.
(d) Harvey cultivated yeast, molds and trichophyta on a medium prepared as follows:
  (1) Prepare: Crushed malt 1 part; distilled water at 70°C. 4 parts.
  (2) Keep 60 minutes at 60°C. in a flask closed with a rubber cork.
  (3) Strain thru muslin.
  (4) Steam 30 minutes.
  (5) Filter.
  (6) Sterilize 20 minutes at 100°C., 3 days.
(e) Harvey added 25.0 cc. of the solution obtained from variant (d) to a liter of water. Further preparation not given.
(f) Harvey added 100.0 g. of malt to a liter of water. Further preparation not given.

References: Wurtz (1897 p. 46), Thoinot and Masselin (1902 p. 30), Tanner (1919 pp. 57, 58), Bezançon (1920 p. 115), Besson (1920 p. 34), Dopter and Sacquépée (1921 p. 120), Harvey (1921–22 pp. 112, 113).

1213. Beijerinck's Malt Wort Solution

Constituents:

1. Water, ditch	1000.0 cc.
2. Malt wort	3.0 cc.
3. Na_2CO_3	1.0 g.
4. Mohr's salt (ammonium ferrous sulphate)	0.2 g.

Preparation:

(1) Add 2, (about 10° Balling) 3, and 4 to 1 liter of ditch water.
(2) A precipitate of calcium and ferrophosphate and carbonate forms.
(3) Shake well so the precipitate is thoroly distributed, and distribute the mixture into a flask or a beaker. Fill the flask full so that there is little surface exposed to air or cover the beaker with a tight fitting glass plate.
(4) Place in the incubator about 25 to 30°C.

Sterilization: Not specified.

Use: To study the reduction of sulphates by *Spirillum desulfuricans*. Author reported that at the end of 5 days there was 35 mg. H_2S formed per liter.

Reference: Beijerinck (1895 p. 54).

1214. Peklo's Infusion Solution

Constituents:

1. Water to	1480.0 cc.
2. Malt	
3. K_2HPO_4	8.0 g.
4. K_2CO_3	6.0 g.

Preparation:
- (1) Add and thoroly mix malt with distilled water.
- (2) Boil for 1 hour and allow to stand at 60°C. for 24 hours.
- (3) Boil again for one hour.
- (4) Filter.
- (5) Dilute the filtrate by the addition of a little distilled water.
- (6) After several sterilizations (times or method not specified) and filtering off the formed precipitate, distribute in sterile Erlenmeyer flasks.
- (7) To 700.0 cc. of (6) add 780.0 g. of distilled water, 8.0 g. K_2HPO_4 and 6.0 g. of K_2CO_3.
- (8) The reaction is strongly alkaline. Sterilization decreases the alkalinity somewhat but the reaction still remains alkaline.

Sterilization: Sterilize (7); method not given.

Use: Cultivation of plant actinomyces.

Reference: Peklo (1910 p. 470).

1215. Bokorny's Malt Infusion Solution

Constituents:

1. Water	500.0 cc.
2. Malt infusion	500.0 cc.
3. Maltose (2.0%)	20.0 g.

Preparation:
- (1) Preparation of malt infusion not given.
- (2) Mix (1) with equal parts of water and add 2.0% maltose.

Sterilization: Not specified.

Use: Cultivation of yeast.

Variants: The author used the following:
- (a) Undiluted malt infusion without any maltose.
- (b) Dilute malt infusion with 3 times its volume of water and add 1.0% maltose.

Reference: Bokorny (1920 p. 30).

1216. Bacto Malt Extract Broth (Dehydrated)

Constituents:

1. Distilled water	1000.0 cc.
2. Malt extract, dehydrated, Bacto	15.0 g.

Preparation:
- (1) Dissolve 15.0 g. Bacto dehydrated malt extract in 1000.0 cc. of distilled water.
- (2) If sterilized for 20 minutes at 15 pounds pressure pH = 4.7±.

Sterilization: Sterilize in the usual manner.

Use: Cultivation of yeast.

Variants: A more concentrated solution may be employed if desired.

Reference: Digestive Ferments Co. (1925 p. 14).

1217. Reddish's Malt Extract Solution

Constituents:

1. Distilled water	900.0 cc.
2. Malt extract	100.0 g.

Preparation:
- (1) Dissolve 100.0 g. dry extract of malt in 900.0 cc. distilled water.
- (2) Make to 8° Kaiser (saccharometer) by adding distilled water (approximately 100.0 cc.).
- (3) Adjust the reaction to +1.5 (1.5% agar may be added and dissolved).
- (4) Autoclave for 15 minutes at 15 pounds pressure.
- (5) Filter thru folded filter paper.
- (6) Tube.

Sterilization: Sterilize at 15 pounds for 10 minutes or 10 pounds for 15 minutes.

Use: Substitute for beer wort.

Reference: Reddish (1919 p. 6).

1218. Park, Williams and Krumwiede's Basal Beer Wort Solution

Constituents:

1. Beerwort.

Preparation:
- (1) Obtain hopped beerwort from the brewery.
- (2) Autoclave.
- (3) Cool.
- (4) Filter.
- (5) Dissolve 2.0% of one of the added nutrients in the filtrate.
- (6) Tube.

Sterilization: Sterilization not specified.

Use: Cultivation of yeast and molds.

Added nutrients: The authors added 2.0% of any desired carbohydrate, alcohol, etc.

Reference: Park, Williams and Krumwiede (1924 p. 134).

1219. Peklo's Beer Wort Solution

Constituents:

1. Distilled water	450.0 cc.
2. Beer wort	550.0 cc.

3. K_2HPO_4	8.0 g.
4. K_2CO_3	6.0 g.

Preparation:

(1) Mix 550.0 cc. of wort beer unhopped and 450.0 cc. of distilled water.

(2) Dissolve 3 and 4 in (1).

Sterilization: Not specified.

Use: Cultivation of plant actinomyces.

Variants: The author used the following solutions:

(a)	1. Distilled water	200.0 cc.
	2. Beer wort	800.0 cc.
	3. K_2HPO_4	15.0 g.
	4. K_2CO_3	2.0 g.
	5. $MgCO_3$	0.0 or 1.8 g.
	or	
	$MgSO_4$	0.0 or 1.8 g.
(b)	1. Distilled water	350.0 cc.
	2. K_2HPO_4	6.0 g.
	3. K_2CO_3	4.5 g.
	4. Beer wort	450.0 cc.
(c)	1. Distilled water	200.0 cc.
	2. Beer wort	800.0 cc.
	3. Na_2CO_3	10.0 g.
(d)	1. Distilled water	200.0 cc.
	2. Beer wort	800.0 cc.
	3. Na_2CO_3	8.0 g.
	4. K_2HPO_4	0.0 or 15.0 g.
	K_2CO_3	0.0 or 4.0 g.
(e)	1. Beer wort	1000.0 cc.
	2. Na_2CO_3	20.0 g.
(f)	1. Distilled water	200.0 cc.
	2. Beer wort	800.0 cc.
	3. Na_2CO_3	2.0 g.
	4. K_2HPO_4	16.0 g.
	5. K_2CO_3	4.0 g.

References: Peklo (1910 pp. 472, 482, 507, 509, 514, 551).

1220. Nakazawa's Beer Wort

Constituents:

1. Beer wort, unhopped.

Preparation: (1) Prepare a 12.0%, unhopped beer wort.

Sterilization: Not specified.

Use: Cultivation of yeast, molds, acetic acid bacteria, etc.

Variants:

(a) Heinze used undiluted beer wort to study nitrogen assimilation of green algae, chlorella, chlorothecium and stichococcus. He reported that nitrogen was not assimilated.

(b) Janke cultivated acetic acid bacteria in hopped lager beer wort of 12.75° Balling. The medium was sterilized in the steamer.

(c) Klimmer, and Park, Williams and Krumwiede boiled and filtered beer wort before sterilization. They cultivated yeast and molds in the medium.

References: Nakazawa (1909 p. 530), Heinze (1906 p. 647), Janke (1916 p. 6), Klimmer (1923 p. 207), Park, Williams and Krumwiede (1924 p. 134).

1221. Peklo's Tartrate Beer Wort Solution

Constituents:

1. Distilled water	350.0 cc.
2. Beer wort	450.0 cc.
3. K_2HPO_4	6.0 g.
4. K_2CO_3	4.5 g.
5. Calcium tartrate	24.0 g.

Preparation:

(1) Mix 350.0 cc. distilled water and 450.0 cc. beer wort.

(2) Dissolve 3 and 4 in (1).

(3) Distribute in 50.0 cc. lots.

(4) To each lot add 1.5 g. of calcium tartrate.

Sterilization: Not specified.

Use: Cultivation of plant actinomyces. Will used a similar medium to cultivate mycoderma.

Variants: Will added 1.0% ammonium tartrate to hopped beer wort of 10.0% B. to cultivate mycoderma.

Reference: Peklo (1910 p. 492), Will (1910 p. 3).

1222. Will's Asparagin Beer Wort Solution

Constituents:

1. Beer wort, hopped	1000.0 cc.
2. Asparagin (0.7%)	7.0 g.

Preparation: (1) Add 0.7% asparagin to hopped beer wort of 10.0% B.

Sterilization: Method not given.

Use: Cultivation of Mycoderma.

Reference: Will (1910 p. 3).

1223. Owen's Cane Juice

Constituents:

1. Cane Juice	1000.0 cc.

Preparation: (1) Distribute cane juice in flasks.

Sterilization: Not specified.

Use: To determine effect of *B. saccharalis* on composition of cane sugar.

Variants:

(a) The author added 1.0% $CaCO_3$ to each flask.

(b) The author sterilized raw sugar cane juice of a pH about 5.0 on each of 3 successive days at 30°C. This medium was used to study the production of gum levan.

Reference: Owen (1916 p. 242), (1923 p. 434).

1224. Owen's Sulphured Cane Juice Solution

Constituents:

1. Sugar cane juice	1000.0 cc.
2. SO_2 (gas)	

Preparation:

(1) Pass SO_2 gas into raw sugar cane juice until 10.0 cc. of the juice require 5.9 cc. of 0.1 normal NaOH to cause neutralization.

(2) pH range from 3.8 to 6.9.

Sterilization: Sterilize on 3 successive days.

Use: To study production of levan by levan forming bacteria isolated from raw sugar. *Bacillus levaniformans*.

Variants: The author added milk of lime to the above medium until the acidity was reduced from 5.9 cc.—0.1 normal NaOH to 0.5 cc. NaOH.

Reference: Owen (1923 p. 434).

1225. Sherman's Corn Juice

Constituents:

1. Corn juice.

Preparation:

(1) Obtain green corn plants in about tosseling stage.

(2) Pressure applied to get juice.

(3) Heat in autoclave a few minutes.

(4) Filter thru filter paper.

(5) Tube.

Sterilization: Method not given.

Use: To show acid production by silage organisms.

Reference: Sherman (1916 p. 449).

1226. Buchanan's Silage Infusion Solution

Constituents:

1. Water, tap	1000.0 cc.
2. Silage	500.0 g.

Preparation:

(1) Boil 500.0 g. of fresh silage for 30 minutes with 1 liter of tap water.

(2) Filter. Wash the silage on the filter with hot water until a liter of the decoction is secured.

(3) Filter.

(4) Distribute in 50.0 cc. lots.

Sterilization: Not specified.

Use: Cultivation of *Monascus purpurens* from silage.

Reference: Buchanan (1910 p. 101).

1227. Reith's Plant Infusion Solution

Constituents:

1. Water, tap	300.0 cc.
2. Wheat	100.0 g.

Preparation:

(1) Soak 100.0 g. wheat in 300.0 g. of tap water for 18 hours.

(2) Autoclave 30 minutes at 15 pounds.

(3) Strain thru cheese cloth.

(4) Adjust to pH = 7.5.

(5) Tube.

Sterilization: Sterilize in the autoclave.

Use: General culture medium. Author reported that the media did not support the growth of Pfeiffer's bacilli unless red blood cells were added, see med. 1228.

Variants: The author treated rye, beans, peas, or hemp in exactly the same manner as wheat above.

Reference: Reith (1923 p. 245).

1228. Reith's Blood Cell Plant Infusion

Enrich medium 1227 by the addition of 2.0% red cells of horse blood.

Use: Cultivation of Pfeiffer bacillus. Gives good growth, but not indol reaction.

Reference: Reith (1923 p. 245).

SUBGROUP I-C. SECTION 16

Liquid media or basal solutions not containing digests, but containing ingredients of animal origin of unknown chemical composition.

A_1.* Only one constituent of unknown chemical composition present.

B_1. Materials of animal origin, exclusive of infusions and extracts employed.

C_1. Containing animal tissues, cells or their derivatives.

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* See page 358 for A_2 and page 357 for B_2.

D$_1$. Brain or other organ employed.
Harrass' Glucose Liver Medium.... 1229
Harrass' Glucose Brain Medium... 1230
Moon's Brain Medium.............. 1231
Weiss' Brain Medium.............. 1232
Zotta's Brain Infusion Solution..... 1233
Harvey's Heart Medium............ 1234
D$_2$. Fish employed.
Harde and Hauser's Fish Medium.. 1235
D$_3$. Bone, horn, etc. employed.
Stoklasa's Bone Meal Solution...... 1236
Remy and Rösing's "Hornspähne" Solution........................ 1237
Cunningham's Hornmeal Solution.. 1238
D$_4$. Meat employed.
Migula's Meat Medium............. 1239
Nevins' Glucose Veal Medium...... 1240
D$_5$. Egg or derivatives employed.
E$_1$. Whole egg employed.
Hueppe's Egg Medium............. 1241
Robertson's Egg Solution (Kahn).. 1242
Harvey's Egg Solution............. 1243
E$_2$. Egg yolk employed.
Nastiukoff's Egg Yolk Solution..... 1244
Harvey's Egg Yolk Solution........ 1245
E$_3$. Egg albumin employed.
F$_1$. Basal solutions; employed with the addition of other constituents.
Hollande and Fumey's Basal Albumin Solution.................. 1246
Waksman's Basal Albumin Solution. 1247
F$_2$. Complete media.
Kent's Glycerol Albumin Solution............................... 1248
Barthel's Albumin Solution......... 1249
Bainbridge's Albumin Solution..... 1250
Castellani's Lactose Albumin Solution (Stitt)...................... 1251
Hogue's Ovomucoid Medium (Hegner and Becker).................. 1252
C$_2$.* Containing animal fluids.
D$_1$. Serum employed.
E$_1$. Basal solutions; employed with the addition of other materials.
Hiss' Basal Serum Solution......... 1253
Leuch's Basal Serum Solution (Klimmer)...................... 1254
E$_2$. Complete media.
F$_1$. Not containing additional organic carbon.
Lorrain-Smith's Alkaline Serum Solution (Heinemann)............ 1255
Klein's Basal Alkaline Serum Solution (Leuch).................... 1256
Martin, Pettit and Vaudremer's Serum Solution.................1256a
Davis' Serum Medium.............. 1257
Marmier's Serum Solution.......... 1258
F$_2$. Containing additional organic carbon.
Boeck's Glucose Serum Solution.... 1259
Sinton's Glucose Serum Solution... 1260
Toyoda's Glucose Serum Solution.. 1261
Marbais' Lactose Serum Solution... 1262
Hiss' Inulin Serum Solution........ 1263
Legroux's Formol Serum Solution.. 1264
Marzinowsky's Citrated Blood Solution......................... 1265
Griffith's Citrated Blood Solution.. 1266
D$_2$.* Blood or its derivatives employed.
E$_1$. Whole blood used.
F$_1$. Citrated.
Besson's Citrated Blood............ 1267
Rogers' Citrated Blood (Stitt)...... 1268
F$_2$. Not citrated.
Buchner's Blood Solution.......... 1269
Davis' Blood Medium.............. 1270
Klimenko's Laked Blood Solution.. 1271
Wurtz and Soppington's Diluted Blood Solution.................. 1272
E$_2$. Defibrinated blood used.
Bass, Foster and Johns' Glucose Blood Solution.................. 1273
Besson's Defibrinated Blood....... 1274
Row's Defibrinated Blood Solution (Harvey)......................... 1275
Carpano's Defibrinated Blood Solution......................... 1276
E$_3$. Blood derivatives used.
Waksman's Basal Fibrin Solution.. 1277
Stoklasa's Glucose Fibrin Solution.. 1278
Beijerinck's Sucrose Fibrin Solution (Percival)........................ 1279
Fischer's Blood Meal Solution...... 1280
Remy and Rösing's Blood Albumin Solution......................... 1281
Klimmer's Hemoglobin Solution.... 1282
D$_3$. Bile employed.
Meyerstein's Bile Medium.......... 1283
Ottolenghi's Nitrate Bile Solution.. 1284
Jackson's Lactose Bile Solution.... 1285
Meyerstein's Glycerol Bile Solution. 1286
D$_4$. Other body fluids employed.
Thoinot and Masselin's Aqueous Humor Medium................. 1287
Chira and Noguchi's Glucose Ascitic Fluid Medium.................. 1288
Sinton's Glucose Body Fluid Solution......................... 1289
C$_3$. Containing animal secretions or excretions.
D$_1$.† Secretions employed.

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* See C$_3$ next column.

* See D$_3$ and D$_4$.

† See page 357 for D

E_1. Skim or whole milk used.

F_1. No indicators added.

Tanner's Milk Powder Solution 1290
Brown and Howe's Milk Solution 1291
Boekhout's Sucrose Milk Solution 1292
Reinsch's Milk Medium 1293

F_2. Indicators added.

G_1. Litmus or its salts used.

Hiss' Basal Litmus Milk Solution 1294
Tanner's Azolitmin Milk Powder Solution 1295
Bacto Litmus Milk (Dehydrated) 1296
Calandra's Picric Acid Litmus Milk Solution 1297
Park and Krumwiede's Litmus Glycerol Milk Solution 1298
Smith's Litmus Milk Solution 1299

G_2. Litmus or its salts not used.

Sherman and Albus' Indicator Milk Solution 1300
Harvey's Brom Cresol Purple Milk Solution 1301
Harvey's China Blue Rosolic Acid Milk Solution 1302
Bacto Purple Milk (Dehydrated) 1303

E_2. Whey used.

F_1. Prepared from a commercial powder.

Bacto Whey Broth (Dehydrated) 1304

F_2. Not prepared from a commercial powder.

G_1. Casein precipitated by an enzyme.

Stutzer and Hartleb's Whey Solution 1305
Emile-Weil's Litmus Whey Solution 1306
Durham's Litmus Whey Solution 1307

G_2. Casein not precipitated by an enzyme.

Giltner's Sour Whey 1308
Bronfenbrenner, Davis and Morishima's China Blue Rosolic Acid Whey Solution 1309
Jouan's Litmus Whey Solution 1310
Petruchsky's Litmus Whey Solution (Grimbert and Legros) 1311

E_3. Casein or its salts used.

F_1. Nutrose specified.

Barsikow's Basal Nutrose Solution 1312
Purwin & McNutt's Basal Nutrose Solution 1313
Thöni and Allemann's Nutrose Solution 1314
Ficker and Hoffmann's Caffine Nutrose Solution 1315
Loeffler's Lactose Nutrose Solution 1316
Hill's Artificial Milk Medium 1317
Doerr's Mannitol Nutrose Solution 1318

F_2. Nutrose not specified.

Whittaker's Lactose Caseinogen Solution 1319
Waksman's Basal Casein Solution 1320
Seliber's Basal Casein Solution (Harvey) 1321
Laxa's Lactic Acid Casein Solution 1322
Baginsky's Casein Solution 1323
Harvey's Alkaline Casein Solution 1324

D_2. Excretions employed.

E_1. Urine used.

Geilinger's Basal Urine Solution 1325
Bokorny's Sucrose Urine Solution 1326
Besson's Urine Medium (Tanner) 1327

E_2. Feces used.

Burri and Stutzer's Nitrate Feces Solution 1328
Dimitroff's Fecal Infusion 1329

B_2. Containing extracts or infusions of materials of animal origin.

C_1. Infusions employed.

D_1. Meat (beef or veal) infusion used.

E_1. Containing no additional organic material.

Dunham's Meat Infusion Solution 1330
Stutzer and Hartleb's Nitrite Infusion Solution 1331

E_2. Containing additional organic material.

Besson's Glucose Infusion Solution 1332
Harvey's Starch Beef Infusion 1333
Beijerinck's Urea Meat Infusion 1334

D_2. Blood (blood clot) infusion used.

Klimmer's Blood Infusion 1335
Szasz's Blood Clot Infusion 1336

D_3. Infusions of special tissue used.

Wellman's Placenta Infusion 1337
Moon's Brain Infusion 1338
Wolbach and Saiki's Liver Infusion 1339
Brieger's Thymus Gland Infusion (Besson) 1340
Nencki, Sieber and Wyznikiewicz's Salivary Gland Infusion 1341
Graham-Smith's Heart Infusion 1342
Robertson and Davis' Heart Infusion Medium 1343

D_4. Infusion of other materials used.

Mereshkowsky's Egg White Infusion 1344
Proca's Spleen Infusion Solution 1345
Harde and Hauser's Fish Infusion 1346
Pergola's Mussel Infusion 1347
Besson's Chicken Infusion (Tanner) 1348

C_2.* Extracts employed.

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* See C_3 page 358.

D_1. Containing no additional organic material.
- Büsgen and Höflich's Meat Extract Solution (Linde) 1349
- Stutzer's Nitrate Meat Extract Solution 1350

D_2. Additional organic nitrogen supplied.
- Zike's Basal Glucose Meat Extract Solution 1351
- Stutzer's Glucose Meat Extract Solution 1352
- Hurler's Succinate Meat Extract Solution 1353
- Blöch's Basal Asparagin Meat Extract Solution 1354
- Kappen's Urea Meat Extract Solution 1355
- Viehoever's Urea Meat Extract Solution 1356
- Kappen's Cyanamide Meat Extract Solution 1357

C_3. Materials other than infusions or extracts employed.
- Homer's Tryptophane Gelatin Solution 1358
- Remy and Rösing's Gelatin Solution. 1359
- Standfuss and Kallert's Bone Jelly Solution 1360
- Berman and Rettger's Gelatin Solution 1361

A_2. More than one constituent of unknown chemical composition present.

B_1. Containing animal cells or tissues.

C_1. Animal fluids added.

D_1. Blood used.
- Fleming's Blood and Minced Meat Medium 1362
- Dean and Nouat's Blood Egg Medium (Cutler) 1363
- Bruschettinis Egg Yolk Blood Infusion Solution 1364

D_2. Serum used.
- Noguchi's Serum Tissue Medium ... 1365
- Hata's Serum Tissue Medium 1366

D_3. Ascitic or other serous fluids used.
- Noguchi's Ascitic Fluid Tissue Medium 1367
- Kligler and Robertson's Ascitic Fluid Egg Medium (Stitt) 1368

C_2. Animal fluids not added.
- Bacto Egg-Meat Medium (Dehydrated) 1369
- Rettger's Egg-Meat Medium 1370
- Besredka's Egg Meat Infusion Medium (Bezançon) 1371

B_2. Not containing animal tissues or cells: containing animal fluids.
- Ficker's Sputum Serum Solution 1372
- Kayser's Blood Bile Solution 1373
- Noguchi's Blood Serum Solution ... 1374
- Berman and Rettger's Casein Meat Extract Medium 1375
- Adams' Coke Milk Medium 1376

1229. Harrass' Glucose Liver Medium

Constituents:

1. Water	1000.0 cc.
2. Liver (veal)	1.0 lb.
3. Glucose (1.0%)	10.0 g.

Preparation:

(1) Grind one pound of calf liver in a meat grinding machine.

(2) Add (1) to 1000.0 cc. of water.

(3) Add 1.0% glucose (or peptone).

(4) Mix thoroly.

(5) Distribute into sterile Erlenmeyer flasks and plug with cotton.

Sterilization: Sterilize for 1.5 to 2 hours in streaming steam.

Use: Cultivate obligate anaerobes.

Reference: Harrass (1906 p. 2237).

1230. Harrass' Glucose Brain Medium

Constituents:

1. Water	1000.0 cc.
2. Brain, veal	1.0 lb.
3. Glucose	10.0 g.

Preparation:

(1) Grind one pound of calf brain in a meat grinding machine.

(2) Add (1) to 1000.0 cc. of water.

(3) Add 1.0% glucose (or 1.0% peptone) to (2).

(4) Mix thoroly.

(5) Distribute into sterile Erlenmeyer flasks and plug with cotton.

Sterilization: Sterilize for 1.5 to 2.0 hours in streaming steam.

Use: Cultivation of obligate anaerobes.

Reference: Harrass (1906 p. 2237).

1231. Moon's Brain Medium

Constituents:

1. Ringer's solution.
2. Brain, dog.

Preparation:

(1) Obtain brain material from normal dog under the most careful aseptic precautions.

(2) Cover brain material with Ringer's solution (see medium 180).
(3) Cover with sterile oil.

Sterilization: Method not given.

Use: Cultivation of Negri bodies.

Reference: Moon (1913 p. 233).

1232. Weiss' Brain Medium

Constituents:

1. Water	1000.0 cc.
2. Sheep brain	2000.0 g.

Preparation:
(1) Mix two parts finely ground sheep brain and one part water.
(2) Do not adjust the reaction.

Sterilization: Sterilize in the autoclave.

Use: To develop *B. botulinus* spores and to study thermal death rates of Clostridia.

Variants: Tanner and McCrea mixed two parts water and one part sheep brain. The pH normally was from 6.8 to 7.0. The medium was sterilized in the autoclave at 15 pounds pressure for 30 minutes in 125 to 150 cc. lots in 250 cc. Erlenmeyer flasks.

References: Weiss (1921 p. 71), Tanner and McCrea (1923 p. 270).

1233. Zotta's Brain Infusion Solution

Constituents:

1. Saline solution (0.75% NaCl)	200.0 cc.
2. Brain (beef)	100.0 g.

Preparation:
(1) Shake 100.0 g. of fresh beef brain freed from its meninges with broken glass until a homogenous mixture is formed.
(2) Add 200.0 cc. of saline solution (0.75% NaCl) to (1) and mix thoroly.
(3) Filter thru gauze.
(4) Heat for 20 minutes.
(5) Tube the supernatant liquid.

Sterilization: Sterilize at 115°C. for 20 minutes. Final pH 6.9.

Use: Cultivation of *Leptomonas pyrrhocoris*.

Reference: Zotta (1923 p. 281).

1234. Harvey's Heart Medium

Constituents:

1. Water	500.0 cc.
2. Heart, beef	500.0 g.

Preparation:
(1) Cut up 500.0 g. of fat-free ox or horse heart.
(2) Add water to just cover the meat.
(3) Cook thoroly and slowly in a closed vessel over a small flame.
(4) Pour off the liquid portion into a beaker.
(5) Mince the solid residue very finely.
(6) Add the minced residue to the liquid in the beaker.
(7) Bring up the weight of the contents of the beaker to 1000.0 g.
(8) Break down any small lumps of meat between the fingers.
(9) Make faintly alkaline to litmus.
(10) Distribute into test tubes.
(11) Cover with a layer of paraffin.

Sterilization: Sterilize 45 minutes at 100°C.

Use: Cultivation of anaerobes.

Variants:
(a) Stitt gave the following method of preparation:
  (1) Grind 250.0 g. of beef heart muscle.
  (2) Suspend (1) in 250.0 cc. of water.
  (3) Heat slowly to cook the meat thoroly.
  (4) Add NaOH to make the reaction neutral to litmus.
  (5) Distribute in test tubes.
  (6) Sterilize in the autoclave.
(b) Park, Williams and Krumwiede prepared the medium as follows:
  (1) Mince 8.0 oz. bullock's heart and grind in a mortar with 8.0 oz. of tap water.
  (2) Cook (time not given).
  (3) Make alkaline.
  (4) Sterilize (method not given).
  (5) Tube.

References: Harvey (1921–22 p. 94), Stitt (1923 p. 42), Park, Williams and Krumwiede (1924 p. 125).

1235. Harde and Hauser's Fish Medium

Constituents:
1. Water.
2. Fish (whiting).

Preparation:
(1) Cut the flesh of a whiting fish in about 1 centimeter cubes and place one cube in a test tube.
(2) Add 8.0 cc. of water to each tube of (1).

Sterilization: Sterilize at 120° for 20 minutes.

Use: General culture medium.

Variants: Kahn prepared the medium as follows:

(1) Free fresh cod fish from bone and skin, chop into small pieces and grind in a mortar.
(2) Add 800.0 cc. tap water for each pound of fish and boil gently for 45 minutes.
(3) Adjust the reaction to pH = 7.2. Prior to testing mix the fish muscle and broth so that one verticle inch of the tissue is present in each tube.
(4) Autoclave at 15 pounds pressure for 20 minutes.
(5) The tubes are sealed with a vaseline cap.

References: Harde and Hauser (1919 p. 1259), Kahn (1922 p. 174).

1236. Stoklasa's Bone Meal Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $CaSO_4$	1.0 g.
3. $MgCl_2$	0.5 g.
4. $FeSO_4$	0.1 g.
5. Bone meal	10.0 g.

Preparation:

(1) Dissolve 2, 3, and 4 in a liter of distilled water.
(2) Place 10.0 g. of finely ground bone meal into a flask. (Bone contains 19.0% H_3PO_4, 5.26% Nitrogen and 1.5% fat).
(3) Add 100.0 cc. of (1) and 800.0 cc. of water to (2).

Sterilization: Method not given.

Use: To study bone decomposition.

Variants: The author used 1.0 g. K_2SO_4 instead of 1.0 g. $CaSO_4$.

Reference: Stoklasa (1900 p. 529), (1911 p. 472).

1237. Remy and Rösing's Horn Shavings Solution

Constituents:

1. Water	1000.0 cc.
2. Horn Shavings	37.5 g.

Preparation:

(1) Dry finely divided "Hornspähne."
(2) Add 0.75 g. of (1) to 20.0 cc. sterile water.
(3) Distribute into small Erlenmeyer flasks.

Sterilization: Sterilize in streaming steam by means of the fractional method.

Use: To study decomposition of organic N materials by soil forms.

Reference: Remy and Rösing (1911 p. 39).

1238. Cunningham's Hornmeal Solution

Constituents:

1. Water	1000.0 cc.
2. Hornmeal (1.0%)	10.0 g.
3. K_2HPO_4 (0.05%)	0.5 g.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Distribute in 10.0 cc. lots in 50.0 cc. flasks or tubes.

Sterilization: Sterilize at 1.5 atmospheres pressure.

Use: To study ammonification by soil and manure forms, *Bac. mycoides*, *Bact. fluorescens*, *Bact. vulgare*, *Bact. coli*, *Sarcina lutea*, *Bac. putrificus*.

Reference: Cunningham (1924 p. 149).

1239. Migula's Meat Medium

Constituents:

1. Water.
2. Meat.

Preparation:

(1) Chop lean beef into small pieces.
(2) Add (1) to Erlenmeyer flasks until a layer about a finger deep is formed.
(3) Add water just to cover the meat layer.

Sterilization: Sterilize the flasks for 30 minutes on each of 3 successive days.

Use: Differentiation of colon and typhoid bacilli. Author reported that the colon bacilli produced a fetid odor while typhoid bacilli did not. Holman cultivated anaerobes, and Besson *Vibrion septique* in similar media.

Variants:

(a) Holman cultivated anaerobes from war wounds on a medium prepared as follows:
  (1) Grind fresh finely chopped fat and fiber free beef muscle in a mortar.
  (2) Mix (1) with equal parts of water.
  (3) Heat slowly to boiling with constant stirring.
  (4) Neutralize to rosalic acid or phenolphthalein.

(5) Tube.
(6) Autoclave for one hour.
(7) Seed by means of a pipette or needle, and mix the inoculum thoroly with the medium.

(b) Holman prepared a similar medium as follows:
(1) Free meat from fat and gross fibers, mince finely. Grind in mortar and mix with equal part of water.
(2) Heat slowly to boiling, stir constantly.
(3) Neutralize or make slightly alkaline while hot to phenolphthalein.
(4) Tube at least 2 inches high and autoclave at 115°C. for 30 minutes.
(5) Just before use, place the tubes in flowing steam for 30 minutes and cool rapidly.
(6) Seed with a pipette, swab or needle and mix material with meat particles.

(c) Bachmann studied the effect of spices on the growth of *Clostridium botulinum*, using the following medium:
(1) Grind hamburger and add enough water to make 100.0 cc.
(2) Add one of the following, cloves, cinnamon, all-spice, ginger, nutmeg, cayenne pepper, white mustard, black mustard in 1.0, 2.0 or 2.5 g. quantity.
(3) Mix thoroly.
(4) Distribute into 6 inch test tubes.
(5) Sterilize in the autoclave.
(6) Inoculate when cool.

He reported that the organisms were not affected by any of the spices used in amounts up to 2.0%. 2.5% retarded the growth of some organisms.

(d) Burke isolated *B. botulinus* in a medium prepared as follows:
(1) Chop 500.0 g. of beef heart or other lean beef with 1000.0 cc. of water.
(2) Bring slowly to a boil, stirring constantly.
(3) Neutralize.
(4) Tube.
(5) Sterilize at 15 pounds pressure for 30 minutes.

(e) Besson cultivated *Vibrion septique* on a medium prepared as follows:
(1) Add 500.00 to 600.00 g. of finely chopped beef to a liter flask.
(2) Add soda solution to neutralize or make slightly alkaline.
(3) Sterilize at 115°C.

(f) Cunningham prepared a similar medium as follows:
(1) Cut up 500.0 g. of fat free bullock's heart into pieces about the size of a pea.
(2) Add 500.0 cc. of water.
(3) Steam until cooked.
(4) Neutralize to turmeric paper (distinctly brown).
(5) Stir well.
(6) Steam for 30 minutes.
(7) Pour off the fluid from the pieces of tissue.
(8) Tube the pieces of heart and just cover them with the extract, taking care to wash down any pieces of tissue which adhere to the sides of the tube.
(9) Sterilize in the autoclave.

References: Migula (1901 p. 24), Holman (1918 p. 125), Bachmann (1918 p. 237), Burke (1919 p. 557), Besson (1920 p. 55), Cunningham (1924 p. 166).

1240. Nevin's Glucose Veal Medium

Constituents:

1. Water	1000.0 cc.
2. Chopped veal	1000.0 g.
3. NaCl	10.0 g.
4. Glucose	40.0 g.

Preparation:
(1) Mix equal parts chopped veal and water.
(2) Add 0.5% NaCl and 2.0% glucose.
(3) Adjust reaction to 0.3% to 0.5% alkaline to phenolphthalein.

Sterilization: Method not given.

Use: Cultivation of *B. botulinus*.

Reference: Nevin (1924 p. 228).

1241. Hueppe's Egg Medium.

Constituents:
1. Eggs.

Preparation:
(1) Wash the eggs thoroly.
(2) Sterilize the shell by dipping in sublimate solution.
(3) Wash in sterile water and dry with sterile cotton.
(4) Make a fine opening in the top of the egg with an instrument heated to glowing.

(5) Inoculate thru the hole with a platinum wire or loop.
(6) Shake well to mix.
(7) Cover the opening with a small piece of sterile paper.
(8) Seal completely with a colloidion membrane.

Sterilization: Given under preparation.

Use: General culture medium.

Variants: Investigators have used slightly different methods in sterilization of the shell, and several have specified that the egg be thoroly shaken before use. The differences are not of sufficient importance to warrant a separate discussion for each investigator.

References: Hueppe (1888 p. 80), Heim (1891 p. 430), Hammerl (1894 p. 155), Abel and Dräer (1895 p. 65), Roux and Rochaix (1911 p. 129), Ball (1919 p. 83), Besson (1920 p. 54), Harvey (1921–22 p. 85).

1242. Robertson's Egg Solution (Kahn)

Constituents:

1. Water	500.0 cc.
2. Egg	

Preparation:
(1) Beat the yolk of one and the whites of 2 eggs in a beaker.
(2) Add 6.0 cc. of N/ NaOH to (1).
(3) Gradually add 500.0 cc. tap water to (2).
(4) Heat very slowly to 95°C. keeping the mixture at this temperature for about an hour.
(5) Filter thru cotton, wool and muslin.
(6) The reaction at this point is about pH = 8.2.
(7) Tube.

Sterilization: Autoclave at 15 pounds pressure for 20 minutes.

Use: Cultivation of spore forming anaerobes. The tubes were sealed with a vaseline cap.

Variants: The author reported better growth in a more acid medium (pH = 7.2) prepared as follows:
(1) Add gradually the yolk of one and the whites of 2 eggs to 500.0 cc. of tap water.
(2) Titrate the mixture so that the reaction is pH = 7.2.
(3) Heat very slowly to 95° and keep at this temperature for an hour.
(4) Autoclave at 15 pounds pressure for 20 minutes.
(5) Some of the albumin will settle to the bottom of the tubes. This does not detract from the value of the medium.
(6) The tubes are sealed with a vaseline cap.

Reference: Kahn (1922 pp. 175, 192).

1243. Harvey's Egg Solution

Constituents:

1. Water	300.0 cc.
2. Egg	

Preparation:
(1) Add the contents of one egg to 300.0 cc. water.
(2) Shake to mix.
(3) Raise the temperature slowly to boiling point with frequent shaking.
(4) Distribute into test tubes.

Sterilization: Sterilize in the autoclave or steamer.

Use: Cultivation of wound organisms and other anaerobes. Also used to cultivate other microörganisms.

Variants: Klimmer prepared the medium as follows for the cultivation of amoeba:
(1) Shake the contents of one egg in a flask containing glass beads.
(2) Add 300.0 cc. of distilled water to (1).
(3) Shake thoroly.
(4) Boil in a water bath for 30 minutes shaking occasionally.
(5) Distribute in 5.0 cc. quantities in tubes.
(6) Sterilize in the autoclave.

Reference: Harvey (1921–22 p. 85), Klimmer (1923 p. 229), Stitt (1923 p. 35), Park, Williams and Krumwiede (1924 p. 126).

1244. Nastiukoff's Egg Yolk Solution.

Constituents:

1. Distilled water	1000.0 cc.
2. Egg yolk	100.0 cc.

Preparation:
(1) Separate the yolk of egg from the white by Bunge's method (allow the whites to roll from a blotting paper containing the egg).
(2) Add 0.5 cc. of a 10% NaOH solution and 100.0 cc. of egg yolk to 1000.0 cc. distilled water.
(3) Place in a flask and steam for 2 hours in a steamer.

(4) Allow to settle for one day.
(5) Filter.
(6) Distribute into test tubes.

Sterilization: Sterilize in the usual manner (method not given).

Use: Cultivation of diphtheria, cholera, influenza and other pathogenic forms.

Variants: Besredka cultivated tubercle bacilli on a medium prepared as follows:

(1) Remove the yolks from 20 eggs (350.0 cc.).
(2) Add one liter of neutral distilled water (neutralize acid if necessary. Indicator not specified).
(3) Prepare a 1.0% soda solution.
(4) Add 175.0 cc. of (3) to (2).
(5) Take a small portion of (4) in a pipette and determine its transparency.
(6) Add (3) to (4) in 1.0 cc. lots until the greatest amount of transparency is obtained, testing as above. (The quantity of soda necessary to be added varies with the yolk.)
(7) Dilute (6) until distilled water has been added to the egg yolk in the ration of 1:20. In this case dilute to 7.0 liters.
(8) Distribute in Roux flasks in 50 to 150.0 cc. lots.
(9) Sterilize at 110°C. for 20 minutes.

References: Nastiukoff (1893 #33 and 34), Besredka (1921 p. 291).

1245. Harvey's Egg Yolk Solution.

Constituents:

1. Egg yolk.

Preparation:

(1) Extract 20.0 g. of egg yolk with 100.0 cc. of 30.0% alcohol for 4 days at 37°C.
(2) Allow to deposit.
(3) Decant the supernatant fluid.

Sterilization: Not specified.

Use: Add 0.25% supernatant fluid to any desired medium. The author treated ascitic fluid in exactly the same manner.

Reference: Harvey (1921–22 p. 121).

1246. Hollande and Fumey's Basal Albumin Solution.

Constituents:

1. Water	1000.0 cc.
2. NaCl	9.0 g.
3. Egg white	
4. Litmus	

Preparation:

(1) Mix the whites of 2 eggs and measure 30.0 cc. into a flask.
(2) Add 70.0 cc. of a physiological salt solution prepared by dissolving 9.0 in a liter of distilled water.
(3) Shake from time to time.
(4) After 15 minutes filter on wet absorbent cotton.
(5) Add 8.0 cc. of a solution containing 4.0 g. soda to 1000.0 cc. distilled water to the filtrate.
(6) Make up the volume of 200.0 cc. by the addition of physiological salt solution (see (2) for preparation).
(7) Heat in the autoclave at 120° for 20 minutes.
(8) Decant.
(9) Distribute in test tubes.
(10) Boil 10.0 g. of litmus cubes in 30.0 cc. ethyl alcohol at 80°C.
(11) Decant, filter and save the filtrate.
(12) Treat the residue on the water bath for 45 minutes with 40.0 cc. distilled water.
(13) Filter and save the filtrate.
(14) Treat the residue with 10.0 or 20.0 cc. of ethyl alcohol at 80°C.
(15) Mix the filtrates and washings.
(16) Neutralize the litmus solution by the addition of 5.0% H_2SO_4 solution.
(17) Dissolve 2.5 g. of one of the added nutrients in 25.0 cc. of (16) by heating.
(18) Allow to cool and immerse strips of bibulous paper in the solution.
(19) Suspend the paper until dryness is reached.
(20) Mix equal parts of ether and alcohol.
(21) Add one part collodion to 9 parts (20).
(22) Dip the paper (19) into (21).
(23) Allow the paper to dry a second time.
(24) Cut the paper into small rectangles 1 by 3 or 4 centimeters.
(25) Add one or two of the rectangles to each tube of (9).

Sterilization: Sterilize for 20 minutes at 118°C.

Use: To study fermentation by dysentery bacillus. The paper is turned red if the sugar is fermented.

Added nutrients: The authors used any desired carbohydrate, alcohol, etc.

Reference: Hollande and Fumey (1917 p. 836).

1247. Waksman's Basal Albumin Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol	30.0 g.
3. K_2HPO_4	1.0 g.
4. KCl	0.5 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.01 g.
7. Egg albumin (powdered)	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and one of the added nutrients in 1.

(2) Dissolve powdered egg albumin in N/10 NaOH, and add to (1).

(3) Tube in 10–12 cc. lots.

Sterilization: Sterilize at 15 pounds for 15 minutes.

Use: To study metabolism of actinomycetes.

Added nutrients: The author added 2.0 g. of one of the following:

$NaNO_3$	$(NH_4)_2SO_4$
$NaNO_2$	$(NH_4)_2CO_3$

Reference: Waksman (1920 p. 3).

1248. Kent's Glycerol Albumin Solution

Constituents:

1. Egg albumin	1000.0 cc.
2. Glycerol (33.0%)	333.0 g.

Preparation:

(1) Add 33.0% or less glycerol to egg albumin.

Sterilization: Not specified.

Use: Cultivation of virus vaccinia. The author reported that the diplo-bacillus vacciniae grew in a cotton-like mass. The glycerol content tended to reduce the growth of staphylococci and other contaminating forms. The medium may be coagulated with heat. (Details not given.)

Reference: Kent (1898 p. 1393).

1249. Barthel's Albumin Solution

Constituents:

1. Egg albumin.

Preparation:

(1) Prepare a 2.0% Na_2CO_3 solution.

(2) Add 8.0 cc. of (1) to Gruber tubes containing sterile egg albumin.

Sterilization: Sterilization of Na_2CO_3 solution not given. Sterilize the egg albumin at 125 to 130°C.

Use: Cultivation of anaerobic bacteria found in milk. The author added 0.1 cc. to 8.0 cc. of Na_2CO_3 solution, and then added the sterile egg albumin. The air was removed.

Reference: Barthel (1910 p. 6).

1250. Bainbridge's Albumin Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Egg albumin	1.0 to 5.0 g.
3. NaCl	5.0 g.
4. Na_2SO_4	1.0 to 2.5 g.
5. $CaCl_2$	trace
6. Potassium phosphate	trace

Preparation:

(1) Dissolve 2 (proteins used were egg albumin, serum protein and alkali-albumin), 3, 4, 5 and 6 in 1.

(2) Add N/100 H_2SO_4 until the medium is very faintly alkaline to extremely sensitive red litmus paper.

(3) After sterilization, distribute in 5.0 cc. quantities.

Sterilization: Sterilize by passing thru a Berkefeld filter.

Use: To study multiplication of bacteria in pure protein solutions. The author reported very slight growth in pure protein media with any of the organisms used.

Reference: Bainbridge (1911 p. 343).

1251. Castellani's Lactose Albumin Solution (Stitt)

Constituents:

1. Water	1000.0 cc.
2. Lactose (1.0%)	10.0 g.
3. Egg albumin (10.0%)	100.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

Sterilization: Method not given.

Use: Cultivation of intestinal protozoa and amoeba. Used also to replace the water of condensation in agar slants.

Reference: Stitt (1923 p. 51).

1252. Hogue's Ovomucoid Medium (Hegner and Becker)

Constituents:

1. Water	600.0 cc.

2. NaCl (0.7%)................ 4.2 g.
3. Egg white

Preparation:

(1) Thoroughly shake up the whites of six eggs with glass beads.
(2) Add (1) to 600.0 cc. of 0.7% saline solution.
(3) Cook for 20 to 30 minutes over a boiling water bath, agitating constantly.
(4) Strain thru cheese cloth.
(5) Filter thru cotton using a suction pump.
(6) Tube in 5.0 cc. lots.

Sterilization.: Sterilize at 15 pounds pressure for 20 minutes.

Use: Cultivation of *Trichomonas hominis* and *Chilomastix mesnili* and other intestinal flagellates.

Reference: Hegner and Becker (1922 p. 18).

1253. Hiss' Basal Serum Solution

Constituents:

1. Water...................... 200.0 cc.
2. Serum...................... 100.0 cc.

Preparation:

(1) Mix two parts water with one part serum and heat to 100°C. for some minutes.
(2) Dissolve one of the added nutrients in (1).

Sterilization: Sterilize at 68°C. for one hour on six consecutive days.

Use: To study fermentation by highly parasitic forms. Hiss used pneumococci and streptococci and reported that pneumococci induced coagulum with glycogen in 24 hours. Streptococci no coagulum. Both organisms produced coagulum with other materials.

Added nutrients and variants:

(a) The author added 3.0 g. of one of the following:

glycogen	galactose
glucose	maltose
lactose	sucrose

(b) The author mixed one part ox serum with two parts distilled water and added 0.1% normal NaOH. Pneumococci caused coagulation after several days in this medium while streptococci did not.

(c) Hiss and Russell diluted 100.0 cc. of beef serum with 200.0 or 300.0 cc. of distilled water, boiled, added 1.0% of any desired carbohydrate, alcohol, etc., added 1.0% of a 5.0% solution of Merck's highly purified litmus solution, tubed and sterilized for 10 minutes on each of 3 successive days in streaming steam.

(d) Elser and Huntoon prepared the medium as follows:

(1) Mix one part sheep serum with two parts distilled water.
(2) Add from 3.0 to 4.5 cc. of a watery solution of Merck's highly sensitized litmus.
(3) Sterilize (method not given).
(4) Prepare a 10.0% solution of one of the following in distilled water:

glucose	mannitol
galactose	dulcitol
levulose	inulin
lactose	dextrin
maltose	sucrose

(5) Sterilize (4) at 100°C. for 10 minutes.
(6) Mix (5) and (3) so that the added nutrient be present in 1.0% concentration.
(7) Tube in sterile tubes.
(8) Incubate for 3 days to detect accidental contamination.

(e) Distaso pepared the medium as follows:

(1) Dilute one volume of beef or sheep serum with three volumes of water.
(2) Sterilize at 120° for 15 minutes.
(3) Dissolve 1.0% of the desired carbohydrate, alcohol, etc., in (2).
(4) Sterilize (method not given).

(f) Krumwiede, Pratt and Kahn gave the following medium for the cultivation of the paratyphoid enteritidis group:

(1) To 400.0 cc. sterile distilled water add 100.0 cc. of sterile horse serum, 5.0 cc. of Andrades indicator and 2.5 cc. of a sterile 25.0% solution of glucose, or any other desired carbohydrate, alcohol etc., in distilled water (final concentration of glucose 0.1%).
(2) Tube to a depth of 4.0 cc. and steam sterilize intermittently on two successive days for 10 minutes. The medium may be prepared from nonsterile materials and sterilized intermittently on 3 successive days.

(g) Roddy prepared the medium as follows:
  (1) Mix 300.0 cc. distilled water with 100.0 cc. of beef blood serum.
  (2) Add 4.0 g. of glucose or any other desired carbohydrate, alcohol, etc., to (1).
  (3) Add a saturated aqueous solution of litmus to give a blue solution.
  (4) Sterilize in the steam sterilizer for 20 minutes on each of 3 successive days.

(h) Baeslack and Keane used a medium prepared as follows for the cultivation of *Spirochaeta pallida* from tissue. The tissue was removed from the patient and pushed into the medium from one-half to two-thirds the length of the tube. Incubate at 37°C. for 3 to 5 days and remove a portion with a sterile pipette on a slide for dark field examination.
  (1) Dilute normal horse serum free from preservatives with sterile distilled water in the proportion of 3:1.
  (2) Fill sterile tubes within an inch from the top with (1) and close the tube with a sterile rubber stopper.
  (3) Heat for an hour in a water bath at 60°C.
  (4) On the following day heat for one hour at 70°C. and on the third day at 70°C. until the medium takes on the consistency of syrup.

(i) Giltner prepared the medium as follows:
  (1) Dilute beef or sheep serum with three times its volume of distilled water.
  (2) Heat in the Arnold for 15 minutes.
  (3) Distribute into desired quantities.
  (4) Add 1.0% of any desired carbohydrate, alcohol, etc. and sufficient litmus to give a deep purple color.
  (5) Sterilize by the fractional method in the steamer.

(j) Abbott gave the following method of preparation:
  (1) Mix one part blood serum with 3 parts distilled water.
  (2) Neutralize (indicator not specified).
  (3) Heat in an Arnold steamer until the mixture becomes opalescent.
  (4) Add 1.0% of a 5.0% aqueous solution of litmus to (3).
  (5) Add 1.0% of any desired carbohydrate, alcohol, etc., to (4).
  (6) Tube.
  (7) Sterilize in the Arnold and allow the steamer to remain uncovered during the process to avoid over heating. Length of time of sterilization not specified.

(k) Harvey prepared the medium as follows:
  (1) Mix one part clear serum (ox) with 3 parts water.
  (2) Steam for 15 minutes (or heat for 20 minutes at 118°C.)
  (3) Add sufficient litmus solution to give a deep blue tint and 1.0% of any desired carbohydrate, alcohol, etc. The litmus solution may be omitted. Heat at 118°C. in step (2) when omitting the litmus.
  (4) Sterilize at 100°C. for 20 minutes on each of 3 successive days, or by filtration.

(l) Gildemeister prepared a medium as follows and used it as a substitute for nutrose in Barsiekow's medium:
  (1) Mix 5.0 to 10.0 cc. of beef serum with 90 to 95.0 cc. of distilled water.
  (2) Sterilize for one hour in a steamer.
  (3) Dissolve 1.0 g. of glucose, lactose or mannitol in 5.0 cc. of Kubel-Tiemann litmus solution.
  (4) Add (3) to (2).
  (5) Distribute in tubes.
  (6) Sterilize on 3 successive days for 15 to 20 minutes each day.

(m) Klimmer used the same basic solution as Gildemeister in variant (l), but used 2.0% mannitol instead of 1.0%, and used 2.0% sucrose and 2.0% maltose also as added nutrients.

(n) Park, Williams and Krumwiede gave the following method of preparation:
  (1) Dilute serum with two or three times its volume of distilled water.
  (2) Sterilize in the Arnold.
  (3) Prepare 10.0 or 20.0% solutions of any desired carbohydrate, alcohol, etc.
  (4) Heat (3) in small containers in the Arnold sterilizer for 30 minutes on 3

successive days. Sterilize the inulin solution in the autoclave.

(5) Add sufficient of (4) to (3) to give a 1.0% concentration of the added nutrient under aseptic conditions. Generally 5.0% glycerol is used instead of 1.0%.

(6) In routine work with glucose, lactose, sucrose, mannitol, and dulcitol it is generally sufficient to add the sugar to the medium and sterilize in the Arnold for 30 minutes on each of 3 successive days.

References: Hiss (1901–05 pp. 325, 327), Hiss and Russel (1903 pp. 289, 295) Elser and Huntoon (1909 p. 404), Distaso (1916 p. 600), Roddy (1917 p. 42), Baeslack and Keane (1920 p. 392), Giltner (1921 p. 383), Krumwiede, Pratt and Kahn (1916–17 p. 357), Abbott (1921 p. 143), Harvey (1921–22 p. 78), Gildemeister (1921–22 p. 76), Klimmer (1923 p. 211), Stitt (1923 p. 36), Park, Williams and Krumwiede (1924 p. 123).

1254. Leuch's Basal Serum Solution (Klimmer)

Constituents:

1. Water	800.0 cc.
2. Serum	180.0 cc.
3. NaCl (0.5%)	5.0 g.
4. NaOH (15.0% soln.)	20.0 cc.

Preparation:

(1) Mix 9 parts serum with 1 part of a 15.0% NaOH solution, and incubate at 37°C. for 2 days.

(2) Make only slightly alkaline to litmus by the addition of 25% HCl.

(3) Dilute one part (2) with 4 parts water and add 0.5% NaCl.

(4) After sterilization, filter.

(5) Dissolve one of the added nutrients in 50.0 cc. of Kubel and Tiemann's litmus solution that has been boiled for 15 minutes.

(6) Heat (5) in a boiling water bath for 6 to 8 minutes.

(7) Filter (6) until clear.

(8) Add (7) to 1000.0 cc. of (4).

(9) Tube.

Sterilization: Method of sterilization of (4) not specified. Sterilize the medium following distribution into tubes on each of 3 successive days for 10 minutes.

Use: To study fermentation.

Added nutrients: Klimmer added one of the following:

glucose	10.0 g.
lactose	10.0 g.
maltose	20.0 g.
sucrose	20.0 g.
mannitol	20.0 g.

Reference: Klimmer (1923 p. 211).

1255. Lorrain-Smith's Alkaline Serum Solution (Heinemann)

Constituents:

1. Serum	1000.0 cc.
2. NaOH (10.0% soln.) (1.0 to 1.5%)	10.0 to 15.0 cc.

Preparation:

(1) Add 1.0 to 1.5% of a 10.0% solution of sodium hydrate to blood serum.

(2) Tube.

Sterilization: Sterilize in the Arnold or Koch's serum inspissator.

Use: General culture medium.

Reference: Heinemann (1905 p. 128).

1256. Klein's Basal Alkaline Serum Solution (Leuch)

Constituents:

1. Water	400.0 cc.
2. Serum	90.0 cc.
3. NaOH (15.0%)	10.0 cc.
4. Litmus	
5. NaCl (0.5%)	2.5 g.

Preparation:

(1) Mix nine parts serum (horse) with one part 15.0% NaOH.

(2) Incubate at 37°C. for two days.

(3) Make only slightly alkaline to litmus by the addition of 25.0% HCl.

(4) Dilute one part serum with four parts water and add 0.5% NaCl.

(5) Sterilize in the autoclave.

(6) Allow to cool and filter.

(7) Boil Kubel and Tiemann's litmus solution for 15 minutes.

(8) Dissolve one of the added nutrients in (7) by boiling 6 to 8 minutes.

(9) Mix (8) and (6) in the ratio of 1:20, while both are warm.

(10) Tube.

Sterilization: Sterilize on each of three successive days for ten minutes each day.

Use: Detection of typhoid-paratyphoid and cholera organisms.

Added nutrients: The author used 1.0% glucose, 1.0% lactose, 2.0% sucrose, 2.0% mannitol, etc.

Variants: The author suggested the use of Czaplewski's alkaline serum (10.0 cc. normal NaOH per 100.0 cc. serum) instead of the alkaline serum given above. Czaplewski's mixture may be sterilized in the autoclave.

Reference: Leuchs (1920 p. 1415), Klein (1920, p. 297).

1256a. Martin, Pettit and Vaudremer's Serum Solution.

Constituents:

1. Physiological salt solution. 1000.0 cc.
2. Serum, bovine.............. 100.0 cc.

Preparation:

(1) Dilute bovine serum 1 to 10 with physiological salt solution.

Sterilization: Not given in the abstract.

Use: Cultivation of *Spirochaeta icterohaemorrhagiae.*

Variants:

(a) Rabbit serum diluted 1:16 proved to be a better medium.

(b) Griffith cultivated *Spirochaeta icterohaemorrhagiae* and other organisms on a medium prepared by diluting one part beef serum with two parts physiological salt solution and heating at 70°C. until the mixture became slightly viscous. He covered the medium with a thin layer of paraffin oil after inoculation.

(c) Harvey mixed 5 parts sterile 0.85% NaCl solution with one part rabbit serum heated at 56°C. for 30 minutes.

(d) Harvey mixed 1 part ox serum heated to 56°C. for 30 minutes with 9 parts sterile 0.85% NaCl solution, or a sterile solution obtained by dissolving 9.2 g. NaCl, 0.05 g. Na_2CO_3, 0.1 g. KCl, 0.1 g. $CaCl_2$ and 10.0 g. sodium citrate in a liter of water. This solution is Locke's solution.

(e) Hogue cultivated *Spirochaeta eurygrata* on a medium prepared as follows:

(1) Dilute serum (best results obtained with pig serum) 1:4.
(2) Tube 0.85% sterile salt solution in 15.0 cc. lots in sterile tubes.
(3) Add 0.3 cc. of (1) to each tube of (2).
(4) A pH = 7.0 gave the best results.
(5) Cover with a layer of paraffin oil.

(f) Stitt cultivated *Balantidium coli* intestinal protozoa and amoeba in a medium prepared as follows:

(1) Mix one part inactivated human blood serum with 16 parts 0.5% salt solution.
(2) The reaction is faintly alkaline to litmus.
(3) Place 8.0 cc. in tubes having a diameter of 10 mm. and a length of 150 mm. giving the medium a depth of about 100 mm.
(4) Sterilization not specified.
(5) Inoculate with 0.1 cc. of undiluted feces containing mucus, by means of the tube capillary pipette into the bottom of the tube.

References: Martin, Pettit and Vaudremer (1917 p. 197), Griffith (1919–20 p. 60), Harvey (1921–22 p. 79), Hogue (1922 p. 619), Stitt (1923 p. 52).

1257. Davis' Serum Medium.

Constituents:

1. Serum, rabbit.

Preparation:

(1) Tube sterile rabbit's blood serum into sterile test tubes.

Sterilization: Method not given.

Use: Used in uncoagulated form by Davis to cultivate the Ducrey bacillus (chancroid bacillus). Other investigators cultivated spirochetes, pneumococci, streptococci, etc., in similar media.

Variants:

(a) Longcope cultivated pneumococci and streptococci in a medium prepared as follows:

(1) Collect 20.0 cc. of blood from the arm vein and allow to clot in a cool place.
(2) Draw off the serum after from 24 to 48 hours.
(3) Use from 2 to 5.0 cc. serum as culture medium.

(b) Schereschewsky heated horse serum at 60°C. until it was brought to a gelatinous consistency, then incubated at 37°C. until partial autolysis was effected. The medium was used for the cultivation of the *syphillus spirochaete.*

(c) Wang tubed serum (ox) in 2.5 cc. quantities and heated at 56°C. for 3 or more successive days until the serum became syrupy. The medium was used in the diagnosis of diphtheria.

(d) Harvey cultivated spirochaetes in sterile rabbit serum heated 30 minutes at 58° to 60°C. covered with a layer of sterile paraffin oil.

References: Davis (1903 p. 405), Longcope (1905 p. 627), Schereschewsky (1909 p. 35), Wang (1919 p. 233), Harvey (1921–22 p. 80).

1258. Marmier's Serum Solution.

Constituents:

1. Water	100.0 cc.
2. Serum (beef)	100.0 cc.

Preparation:

(1) Mix equal parts of beef serum and water.

(2) Make slightly alkaline by the addition of soda.

Sterilization: Sterilize at 115° in the coagulator (time not specified).

Use: Anthrax toxin production by anthrax bacilli.

Reference: Marmier (1895 p. 569).

1259. Boeck's Glucose Serum Solution.

Constituents:

1. Distilled water	1000.0 cc.
2. $NaHCO_3$	0.2 g.
3. KCl	0.42 g.
4. $CaCl_2$	0.24 g.
5. NaCl	9.0 g.
6. Glucose	2.5 g.
7. Serum (human, sheep or horse)	250.0 cc.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Mix aseptically one part sterile serum (preferably human) with 4 parts sterile (1). The usual reaction of the mixture is 0.2% alkaline to phenolphthalein.

(3) Tube in 5.0 cc. lots in sterile tubes.

(4) Incubate at 37° over night to test sterility.

Sterilization: Method of sterilization of (1) not given.

Use: Cultivation of *Chilomastix mesnili*, a parasite in the small intestine of man.

Reference: Boeck (1921 p. 159).

1260. Sinton's Glucose Serum Solution

Constituents:

1. Serum, horse	1000.0 cc.
2. Glucose (50.0% soln.)	15.0 cc.

Preparation:

(1) Add 1.5 cc. of a 50.0% sterile glucose solution to each 100.0 cc. of aseptically collected horse serum.

Sterilization: Not specified.

Use: Cultivation of *Spirochaeta carteri* causing Indian relapsing fever.

Reference: Sinton (1923–24 p. 826).

1261. Toyoda's Glucose Serum Solution

Constituents:

1. Serum, dog	1000.0 cc.
2. Glucose	10.0 g.
3. Sodium citrate solution	
4. Physiological salt solution	

Preparation:

(1) To each 10.0 cc. of healthy young dog serum add 0.2 cc. of a 50.0% sterile glucose solution and 0.3 cc. of a 2.0% sterile sodium citrate in 0.85% NaCl solution.

(2) Pipette the serum in small narrow test tubes 5 cm. high and inactivate the serum at 45°C. for one hour.

Sterilization: Not specified.

Use: Cultivation of *Babesia cannis*

Reference: Toyoda (1913 p. 76).

1262. Marbais' Lactose Serum Solution

Constituents:

1. Distilled water	300.0 cc.
2. Serum	100.0 cc.
3. Lactose	8.0 g.
4. Litmus	

Preparation:

(1) Add one volume of clear serum (man or animal, preferably horse or sheep) to three volumes of neutral distilled water.

(2) Add sufficient lactose to make 2.0 g. lactose per 100.0 cc. of mixture.

(3) Mix well and filter thru paper.

(4) Tube in 7 or 8.0 cc. lots.

(5) Add 0.1 cc. of a sterile litmus solution prepared according to Besson (reference or method not given) to each sterile tube.

(6) Incubate for 24 hours to test sterility.

Sterilization: Sterilize (4) at 120°C. for 15 minutes.

Use: Differentiation of colon-typhoid bacilli. Author reported that typhoid cause coagulation after 24 hours, color was blue-violet. Paratyphoid A clouded the medium, and the color was lilac. Paratyphoid B gave a red color after 5 or 6 hours incubation, then changed to original color. *B. coli* coagulated and decolorized the medium.

Reference: Marbais (1918 p. 602).

1263. Hiss' Inulin Serum Solution

Constituents:

1. Distilled water	200.0 cc.
2. Beef serum	100.0 cc.
3. Inulin (pure) (1.0%)	3.0 g.
4. Litmus (5.0% soln.)	3.0 cc.

Preparation:

(1) Mix two parts distilled water with one part fresh clear beef serum.

(2) Add 1.0% pure inulin, and 3.0 cc. of 5.0% solution Merck's highly purified litmus.

Sterilization: Sterilize at 100°C. for 10 minutes (if inulin contains heat resisting spores, autoclave at 10 to 15 pounds pressure for 15 minutes.)

Use: Differentiation of pneumococci and streptococci. Author reported that pneumococci. cultures coagulated the serum, streptococci did not.

Variants:

(a) Hiss omitted the litmus.

(b) Hiss prepared a similar medium as follows:

  (1) Add 4.0 g. powdered starch to 400.0 cc. water, and boil for 30 minutes. Allow to stand over night.

  (2) Obtain, next morning, the clear fluid by pipetting off the water from the starch particles.

  (3) Add one part serum to two parts of the supernatant fluid from (2).

  (4) Sterilize at 68°C. for 1 hour on 6 consecutive days.

(c) Hiss heated the beef serum and water at 100°C. for several minutes and then added the inulin and litmus.

(d) Harvey prepared the medium as follows:

  (1) Mix 1 part ox serum with 3 parts water.

  (2) Steam 15 minutes.

  (3) Add sufficient litmus solution to give a blue color.

  (4) Prepare a 10.0% solution of inulin.

  (5) Sterilize (4) at 15 pounds pressure for 15 minutes.

  (6) Mix 1 part (5) with 10 parts (3).

  (7) Sterilize on each of 3 successive days for 20 minutes at 100°C.

Reference: Hiss (1901–05 pp. 324, 330), Harvey (1921–22 p. 112).

1264. Legroux's Formol Serum Solution

Constituents:

1. Distilled water	1200.0 cc.
2. Serum (beef or horse)	600.0 cc.
3. Formol	1.0 cc.

Preparation:

(1) Mix 600.0 cc. of beef or horse serum with 1.0 cc. of commercial formol.

(2) Dilute (1) with 1200.0 cc. distilled water.

(3) Mix well.

(4) The reaction is acid to methyl red.

(5) Distribute in tubes or flasks.

Sterilization: Sterilize in the autoclave at 112 to 115° for varying lengths of time depending on the size of the container.

Use: Enrichment of meningococci. The author reported that the formol kept the medium from coagulating during sterilization. It also aided in giving a clear medium.

Reference: Legroux (1920 p. 466).

1265. Marzinowsky's Citrated Blood Solution

Constituents:

1. Water	100.0 cc.
2. Sodium citrate (10.0%)	10.0 g.
3. Blood, horse	

Preparation:

(1) Prepare a 10.0% watery solution of sodium citrate.

(2) Distribute in 1.5 to 2.0 cc. lots in test tubes.

(3) Add 10.0 cc. of horse blood to each tube of sterile (2). The blood is taken directly from a vein.

Sterilization: Sterilize (2); method not given.

Use: Cultivation of *Piroplasma equi* and other piroplasma.

Reference: Marzinowsky (1908–09 p. 419).

1266. Griffith's Citrated Blood Solution

Constituents:

1. Blood, citrated (horse, beef, rabbit)	100.0 cc.
2. Physiological salt solution	200.0 cc.

Preparation:

(1) Mix 1 part citrated blood (horse, beef or rabbit) with 2 parts physiological saline.

(2) Heat until semi-gelatinous.

Sterilization: Not specified.

Use: Cultivation of *Spirochaeta icterohaemorrhagiae.* The author inoculated the medium with infected heart blood or tissue fragment, and covered the medium with a thin layer of paraffin oil.

Reference: Griffith (1919–20 p. 61).

1267. Besson's Citrated Blood

Constituents:

1. Water	1000.0 cc.
2. NaCl	8.0 g.
3. Sodium citrate	15.0 g.
4. Blood	

Preparation:

(1) Dissolve 8.0 g. NaCl and 15.0 g. of sodium citrate in 1000.0 cc. of water.

(2) Mix 20.0 cc. of sterile (1) with 80.0 cc. of blood obtained under aseptic conditions.

Sterilization: Sterilize (1); method not given

Reference: Besson (1920 p. 34).

1268. Rogers' Citrated Blood (Stitt)

Constituents:

1. Blood.
2. Sodium citrate (10.0%).
3. Citric acid.

Preparation:

(1) Place 1.0 or 2.0 cc. of a sterile 10.0% sodium citrate slightly acidified with citric acid into the barrel of a syringe and aspirate splenic blood directly into it.

Sterilization: Not specified.

Use: Cultivation of Leishmania.

Reference: Stitt (1923 p. 52).

1269. Buchner's Blood Solution

Constituents:

1. Water	1000.0 cc.
2. Blood	100.0 cc.
3. Sucrose (10.0%)	100.0 g.

Preparation:

(1) Prepare a 10.0% solution of cane sugar. (Reaction may be slightly alkaline.)

(2) Add 0.1 volume of blood to sterile (1).

Sterilization: Sterilize (1) by heating at 55°C.

Use: To show influence of sugar on development of anthrax and other organisms. Author reported that 10.0% sugar increased the bacterial count.

Variants: The author omitted the sucrose or used 40.0% instead of 10.0%.

Reference: Buchner (1890 p. 67).

1270. Davis' Blood Medium

Constituents:

1. Blood, human.

Preparation:

(1) Prick the extensor surface of the thumb just back of the base of the nail, the skin having been previously cleansed with soap and water and alcohol.

(2) Applying a tourniquet at the base of the thumb just tight enough to obstruct the venous flow, a considerable quantity of blood may be obtained.

(3) The blood is drawn up by capillary attraction into a small glass tube, one end of which has been drawn out to a very fine caliber over the Bunsen flame, and the whole tube sterilized by the same means.

(4) When about 0.2 cc. of blood has flowed into the tube the capillary end may be sealed in the flame, and the other end plugged with cotton, giving a miniature culture tube.

(5) The medium is nearly always sterile when obtained in the manner indicated.

Sterilization: Given in the preparation.

Use: Cultivation of Ducrey bacillus (chancroid bacillus), pneumococci and streptococci.

Variants: Stitt reported that pneumococci and streptococci maintained their virulence in a medium prepared as follows:

(1) Obtain rabbit or human blood under aseptic conditions and preserve (whole blood) in small test tubes.

(2) Heat for 30 minutes at 56°C. to inactivate the blood.

References: Davis (1903 p. 405), Stitt (1923 p. 44).

1271. Klimenko's Laked Blood Solution

Constituents:

1. Distilled water	900.0 cc.
2. Blood	10.0 cc.

Preparation:
- (1) Mix 10 parts blood and 90 parts sterile distilled water.
- (2) Tube.

Sterilization: Not specified.

Use: Cultivation of whooping cough bacillus. Author reported that the organisms grew first on the surface of the medium, then on the bottom; after several days the whole tube became turbid and was finally colored dark brown.

Reference: Klimenko (1909 p. 312).

1272. Wurtz and Sappington's Diluted Blood Solution

Constituents:

1. Water	18.0 cc.
2. Blood	12.0 cc.

Preparation:
- (1) Rub the skin at the bend of the elbow with a pledget of cotton soaked in alcohol.
- (2) Take the blood with a large sized Wassermann needle kept dry—sterilized in a test tube.
- (3) Distend the vein by a knotted piece of rubber tubing above, and the patients clinched fist below.
- (4) Plunge the needle into the vein and allow about 10.0 cc. of blood to run into a dry sterile test tube. This washes out the needle and lessens the chance of contamination.
- (5) Run about 12.0 cc. of the blood then into a tube containing 18.0 cc. of sterile water.

Sterilization: Sterilization given under preparation.

Use: Blood culture. Author reported that this method was especially good in case of pneumococcic and streptococcic infections.

Reference: Wurtz and Sappington (1918 p. 373).

1273. Bass, Foster and Johns' Glucose Blood Solution

Constituents:

1. Blood (malarial)	100.0 cc.
2. Glucose	0.5 g.

Preparation:
- (1) Draw the blood from the patients vein at the bend of the elbow.
- (2) Add 0.1 cc. of 50.0% dextrose solution for every 10.0 cc. of blood drawn.
- (3) Defibrinate the blood by gently whipping or stirring with a glass rod. Avoid air bubbles.
- (4) After defibrination, plug the tube with a fresh sterile plug.
- (5) The column of blood must be from one to two inches thick. This gives a column of serum ½ to 1 inch thick.

Sterilization: Not specified.

Use: Cultivation of malarial plasmodia.

Variants:
- (a) Harvey prepared the medium as follows:
  - (1) Add 15.0 cc. aspirated malarial blood to a centrifuge tube containing 0.1 cc. 50.0% glucose.
  - (2) Defibrinate the blood-glucose mixture with a glass rod.
  - (3) Centrifuge.
  - (4) Observe the development of parasites at 41°C. under anaerobic conditions.
- (b) Stitt gave the following method of preparation:
  - (1) Place 0.1 cc. of a 50.0% glucose solution in a centrifuge tube.
  - (2) Add from 10.0 to 20.0 cc. of malarial patients blood to each tube of (1).
  - (3) Defibrinate the blood by extending a piece of glass rod or piece of tubing into the bottom of the centrifuge tube.
  - (4) Centrifuge.
  - (5) There should be at least one inch of serum above the cell sediment.
- (c) Park, Williams and Krumwiede used the following method:
  - (1) Defibrinate carefully 10.0 cc. of blood taken from a malarial patient.
  - (2) Place in small test tubes in 1.0 cc. amounts.
  - (3) One per cent of a 50 per cent solution of dextrose is added to each small test tube before adding the blood.
  - (4) The red corpuscles settle so that a 0.5 cm. layer of serum is left above them.

References: Bass, Foster and Johns (1912 p. 570), Harvey (1921–22 p. 72), Stitt (1923 p. 53), Park, Williams and Krumwiede (1924 p. 133).

1274. Besson's Defibrinated Blood

Constituents:

1. Blood.

Preparation:

(1) Collect blood under aseptic conditions in a sterile flask containing glass beads.
(2) Shake thoroly for 10 minutes.
(3) Remove the liquid from the fibrin by asparating.
(4) Distribute the fluid in sterile test tubes.

Sterilization: Not specified.

Variants: Harvey did not separate the fluid from the fibrin and incubated for 48 hours to test sterility.

References: Besson (1920 p. 34), Harvey (1921–22 p. 73).

1275. Row's Defibrinated Blood Solution (Harvey)

Constituents:

1. Distilled water	1000.0 cc.
2. Blood, defibrinated	100.0 cc.
3. NaCl (1.2% solution)	2200.0 cc.

Preparation:

(1) Defibrinated human or rabbit blood.
(2) Add 10 times its volume distilled water.
(3) Add 1 volume of the laked blood thus obtained to 2 volumes 1.2% sterile sodium chloride solution.

Sterilization: Not specified.

Use: Cultivation of Leishmania.

References: Harvey (1921–22 p. 72), Stitt (1923 p. 52).

1276. Carpano's Defibrinated Blood Solution

Constituents:

1. Distilled water	100.0 cc.
2. NaCl (c.p.)	7.0 g.
3. Sodium citrate (c.p.)	7.0 g.
4. Defibrinated horse blood	

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Distribute in 1.0 cc. lots into test tubes.
(3) Remove blood under aseptic conditions from the jugular vein of a horse.
(4) Defibrinate (3) and store in the ice box if not to be used at once.
(5) Add 9.0 cc. of (4) to each tube of sterile (2) under strictly aseptic conditions.
(6) Mix well and place in an incubator at 25°C. for 24 hours. At the end of this time the mixture is clear.

Sterilization: Sterilize (2) carefully. (Method not given.)

Use: Cultivation of *Babesia caballi* and *Nuttallia equi.* To inoculate carefully place the end of a sterile fine pointed pipette containing infected blood into the red blood cells in the bottom of the tube, and allow the infected blood to run into the tube.

Reference: Carpano (1914 p. 43).

1277. Waksman's Basal Fibrin Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol	30.0 g.
3. K_2HPO_4	1.0 g.
4. KCl	0.5 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.01 g.
7. Fibrin	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and one of the added nutrients in 1.
(2) Tube in 10–12 cc. lots.
(3) Add small pieces of fibrin to each tube.

Sterilization: Sterilize at 15 pounds for 15 minutes.

Use: To study metabolism of actinomycetes.

Added nutrients: The author added 2.0 g. of one of the following:

$NaNO_3$	$(NH_5)_2SO_4$
$NaNO_2$	$(NH_4)_2CO_3$

Reference: Waksman (1920 p. 3).

1278. Stoklasa's Glucose Fibrin Solution

Constituents:

1. Water	1000.0 cc.
2. Sodium phosphate	0.5 g.
3. K_2SO_4	0.2 g.
4. $MgCl_2$	0.05 g.
5. Glucose	2.5 g.

6. $CaCO_3$	7.5 g.
7. Fibrin	125.0 g.

Preparation:

(1) Dissolve 0.5 g. sodium phosphate, 0.2 g. K_2SO_4, and 0.05 g. $MgCl_2$ in 500.0 cc. water.
(2) Dissolve 2.5 g. glucose in 500.0 cc. water.
(3) Mix 200.0 cc. (1) and 120.0 cc. of (2).
(4) Dissolve 7.5 g. $CaCO_3$ in (3).
(5) Distribute in 200.0 cc. lots.
(6) Add 25.0 g. of fibrin from blood to each flask.

Sterilization: Method not given.

Use: To study utilization of nitrogen by *Bacillus megatherium*, (*Bacillus Ellenbachii*) or "Alinit." The author reported that more nitrogen was found to be in solution after incubation.

Variants: The author also gave the following method of preparation:

(1) Dissolve 0.5 g. sodium phosphate, 0.2 g. potassium sulphate and 0.05 g. magnesium chloride in 500.0 cc. water.
(2) Dissolve 2.5 g. glucose in 500.0 cc. water.
(3) Mix 400.0 cc. (1) with 240.0 cc. of (2).
(4) Dissolve 6 in (3).
(5) Dilute to 4 liters.
(6) Place 1 liter in a large flask and add 40.0 g. fibrin to each flask.

Reference: Stoklasa (1898 p. 288).

1279. Beijerinck's Sucrose Fibrin Solution (Percival)

Constituents:

1. Water	1000.0 cc.
2. Cane sugar	50.0 g.
3. Fibrin	50.0 g.
4. $CaCO_3$ (precipitated)	3.0 g.
5. Sodium phosphate	0.5 g.
6. $MgSO_4$	0.5 g.
7. NaCl	0.5 g.

Preparation:

(1) Boil 2, 3, 4, 5, 6 and 7 in 1 briskly for 2 or 3 minutes. The fibrin is to be finely ground.

Sterilization: Not specified.

Use: Isolation of *Granulobacillus saccharobutyricus*. The medium was inoculated by the addition of 10.0 to 15.0 g. of fresh garden soil while still boiling.

Reference: Percival (1920 p. 288).

1280. Fischer's Blood Meal Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Blood meal	10.0 g.
3. KH_2PO_4	1.0 g.
4. $CaCl_2$	0.1 g.
5. $MgSO_4$	0.3 g.
6. NaCl	0.1 g.
7. Fe_2Cl_6	0.01 g.

Preparation:

(1) Dissolve 3, 4, 5, 6 and 7 in a liter of water.
(2) Distribute in 200.0 cc. lots.
(3) Add 2.0 g. of blood meal to each flask of (2).

Sterilization: Sterilize in the autoclave.

Use: To study decomposition of organic nitrogen by soil forms. Each flask was inoculated with 25.0 cc. of a soil suspension prepared from 100.0 g. soil in 500.0 cc. of water. The author reported that the flask containing a trace of acid gave the most ammonia while the one with a trace of alkali showed least ammonia production.

Variants: The author gave the following variants:

(a) Dissolve 2.0 g. of blood meal in 200.0 cc. of water. Add no salts.
(b) Dissolve 2.0 g. of blood meal in 200.0 cc. of water, no salts, and add 5.0 cc. of a 1 to 10,000 diluted 0.5 normal H_2SO_4.
(c) Dissolve 2.0 g. of blood meal in 200.0 cc. of water, no salts, and add 5.0 cc. of a 1 to 10,000 diluted 0.5 normal NaOH.

Reference: Fischer (1909 p. 65).

1281. Remy and Rösing's Blood Albumin Solution

Constituents:

1. Water	1000.0 cc.
2. Blood albumin	50.0 g.

Preparation:

(1) Grind dry blood albumin fine.
(2) Add 1.0 g. sterile (1) to 20.0 cc. of sterile water.
(3) Distribute into small Erlenmeyer flasks.

Sterilization: Sterilize (1) twice at 100°C. for 30 minutes. Method of sterilization of the water not given. Sterilize the finished medium in the steamer, using the fractional method.

Use: To study decomposition of organic N materials by soil forms. Author reported little change in total nitrogen content after 12 days when inoculated with soil.

Reference: Remy and Rösing (1911 p. 39).

1282. Klimmer's Hemoglobin Solution

Constituents:

1. Blood	1000.0 cc.
2. Physiological salt solution	1000.0 cc.

Preparation:

(1) Collect pigeon blood under aseptic conditions and add to an equal volume of physiological salt (0.85%) solution.
(2) Mix well and centrifuge, or allow to stand in the cold for 24 hours.
(3) Thoroly wash the red residue twice more with sterile NaCl solution.
(4) Dissolve the hemoglobin with a trace of ether.
(5) Evaporate the ether at 30°C.
(6) Separate the hemoglobin from the stromata by passing thru a bacterial filter.

Sterilization: Method of sterilization of salt solution not specified.

Use: This hemoglobin solution is added to other media which is used for the cultivation of pneumococci, vibrio, etc. One cubic centimeter of this solution is added to 8.0 cc. of media, or this solution is streaked on solid media.

Variants: The author also used commercial hemoglobin. This solution was prepared as follows:

(1) Mix 10.0 g. of commercial hemoglobin with 90.0 cc. of distilled water.
(2) Add about 10.0 cc. of 10.0% KOH.
(3) Sterilize in the steamer.

Reference: Klimmer (1923 p. 196).

1283. Meyerstein's Bile Medium

Constituents:

1. Bile, beef.

Preparation:

(1) Evaporate beef bile with animal charcoal to dryness on a water bath.
(2) Take up the residue in alcohol.
(3) Filter.
(4) Add quite a lot of ether to the somewhat concentrated filtrate. This causes the bile acid to precipitate out in silky crystals—so-called crystalline bile.
(5) Dry and pulverize the crystals.
(6) Add one or two knife points of (5) to sterile test tubes.

Sterilization: Medium sterilized during the preparation.

Use: Enrichment of typhoid bacilli from patients blood. Diagnosis of typhoid fever. The author added 2.0 to 4.0 cc. of the patients blood to each tube of (6).

Variants: The following authors have prepared media in the manner indicated.

(a) Kayser
  (1) Secure normal beef bile immediately after the death of an animal in large sterile flasks.
  (2) Distribute in amounts of at least 5.0 cc. in test tubes.
  (3) Sterilize at 110°.
  (4) Put 2.5 cc. of blood of patient into this at bedside.
(b) Bezançon.
  (1) Collect bile at a slaughter house.
  (2) Sterilize at 100°C.
  (3) Allow to settle.
  (4) Decant the clear liquid.
  (5) Tube in 3.0 cc. quantities in sterile tubes.
  (6) Sterilize at 105°C. for 20 minutes.
(c) Klimmer.
  (1) Puncture the gall bladder of a beef with a knife, and collect the bile.
  (2) Boil (1) in a steamer for 15 minutes.
  (3) Distribute in 5.0 cc. quantities in test tubes.
  (4) Steam on each of 3 successive days for 20 to 30 minutes.

References: Meyerstein (1906 p. 1864), Kayser (1906 pp. 823–826), Harvey (1921–22 p. 89), Bezançon (1920 p. 122), Klimmer (1923 p. 202), Stitt (1923 p. 47).

1284. Ottolenghi's Nitrate Bile Solution

Constituents:

1. Bile	1000.0 cc.
2. Na_2CO_3 (10.0% soln.) (3.0%)	30.0 cc.
3. KNO_3 (1.0%)	1.0 g.

Preparation:

(1) Filter fresh ox bile thru a paper.
(2) Add 3.0% of a 10.0% watery solution

of crystalline Na_2CO_3 and 0.1% KNO_3 to (1).
(3) Distribute in 5.0 cc. lots.

Sterilization: Sterilize in the autoclave for 15 to 20 minutes at 0.5 atmosphere.

Use: Enrichment medium for cholera vibrio.

Reference: Ottolenghi (1911 p. 370).

1285. Jackson's Lactose Bile Solution

Constituents:

1. Bile, ox	1000.0 cc.
2. Lactose (1.0%)	10.0 g.

Preparation:
(1) Draw bile, and sterilize undiluted. It may be kept in stock in this manner.
(2) When ready for use, decant or filter.
(3) Add 1.0% lactose that has previously been dissolved in small (amount not given) amount of water.
(4) Mix (2) and (3) and tube.

Sterilization: Sterilize in autoclave for 30 minutes at 15 pounds pressure.

Use: Enrichment medium used in water analysis.

Variants: Stitt reported that a 15 to 20.0% solution of a good quality of inspissated ox bile may be substituted for fresh ox bile.

References: Jackson (1907 p. 31), Stitt (1923 p. 47).

1286. Meyerstein's Glycerol Bile Solution

Constituents:
1. Bile, ox.
2. Glycerol.

Preparation:
(1) Evaporate ox gall with animal charcoal on a water bath.
(2) Take up the residue in alcohol.
(3) Filter.
(4) Add quite a lot of ether to the somewhat concentrated filtrate. This causes the bile acid to precipitate out in silky crystals—so-called crystalline bile.
(5) Dry and pulverize the crystals.
(6) Dissolve about 30.0 or 40.0% of (5) in glycerol by heating.
(7) Place several drops of (6) in test tubes.

Sterilization: Medium sterilized during the preparation.

Use: Enrichment of typhoid bacilli from patients blood. Add 1.0 cc. of patients blood to each tube of (6).

Reference: Meyerstein (1906 p. 1864).

1287. Thoinot and Masselin's Aqueous Humor Medium

Constituents:
1. Aqueous humor.

Preparation:
(1) Obtain the aqueous humor from the eye of an animal by searing the cornea with a hot piece of iron and then puncturing the cornea with a pipette.
(2) Distribute as desired.

Sterilization: Not specified.

Use: Cultivation of anthrax bacilli.

Reference: Thoinot and Masselin (1902 p. 28).

1288. Ohira and Noguchi's Glucose Ascitic Fluid Solution

Constituents:

1. Ringer's Solution	1000.0 cc.
2. Ascitic fluid	1000.0 cc.

Preparation:
(1) Mix equal portions of ascitic fluid and Ringer's solution. (See medium 180).
(2) Do not adjust the reaction.

Sterilization: Method not given.

Use: Cultivation of Trichomonas of the human mouth. (*Tetratrichomonas hominis*).

Reference: Ohira and Noguchi (1917 p. 342).

1289. Sinton's Glucose Body Fluid Solution

Constituents:

1. Ascitic fluid	100.0 cc.
2. Glucose (50.0% soln.).	1.5 to 2.0 cc.

Preparation:
(1) Draw off ascitic fluid with strict aseptic precautions into sterile flasks until each contains 100.0 cc.
(2) Add 1.5 to 2.0 cc. of 50.0% sterile glucose solution to each flask.
(3) Heat for 30 minutes at 56°C. to kill as much complement as possible.

Sterilization: Method of sterilization of 50.0% glucose solution not given.

Use: Cultivation of *Plasmodium falciparum.*

Variants: The author used hydrocele fluid instead of ascitic fluid.

Reference: Sinton (1922–23 p. 205).

1290. Tanner's Milk Powder Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Milk Powder, Merrell-Soule	100.0 g.

Preparation:

(1) Add 2 to 1.
(2) Beat with an egg beater.
(3) Filter.
(4) Distribute in 75.0 cc. quantities.

Sterilization: Sterilize in the steamer.

Use: General culture medium. Author cultivated green fluorescent bacteria from water.

Reference: Tanner (1918 p. 83), (1919 p. 71).

1291. Brown and Howe's Milk Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Milk (skim)	500.0 cc.
3. Sodium citrate	4.0 g.

Preparation:

(1) Add 500.0 cc. of milk to a liter of water.
(2) Add 4.0 g. of sodium citrate and allow to stand for one hour.
(3) Filter.
(4) Adjust to pH 6.8.
(5) Tube.

Sterilization: Sterilize in Arnold. (A precipitate appears when heated but disappears when cool).

Use: General culture medium.

Variants: The author used 4.0 g. sodium oxalate instead of 4.0 g. sodium citrate. Citrated milk was found to be more satisfactory than oxalated milk.

Reference: Brown and Howe (1922 p. 512).

1292. Boekhout's Sucrose Milk Solution

Constituents:

1. Milk	1000.0 cc.
2. Sucrose (8.0%)	80.0 g.

Preparation:

(1) Dissolve 8.0% sucrose in milk.

Sterilization: Not specified.

Use: Cultivation of dextran formers. (*Streptococcus hornensis*). Organism may be isolated from flowers, honey and bees.

Reference: Boekhout (1900 p. 162).

1293. Reinsch's Milk Medium

Constituents:

1. Milk.

Preparation:

(1) Place 500.0 cc. of fresh cow milk in a separatory funnel and add 1.0 g. NaOH (2.5 cc. solution of 400.0 g. NaOH in a liter).
(2) Shake well and allow to stand at about 18°C. for 48 hours.
(3) Remove the nearly transparent milk from the bottom of the funnel and add it to a second separatory funnel.
(4) Add 250.0 cc. ether and shake well.
(5) Allow to stand for 48 hours.
(6) Place the opalescent liquid now in a large sterile flask, plug with cotton and heat to 50°C.
(7) Place under the receiver of a water suction pump for 3 or 4 hours until all the ether is evaporated.

Sterilization: Given under preparation.

Use: General culture medium. Medium is fat free.

Variants:

(a) Migula gave the following method of preparation:
  (1) Obtain milk under aseptic conditions in a sterile flask.
  (2) Add 2.0 cc. of chloroform to 100.0 cc. of (1).
  (3) Seal the flask with a rubber stopper and shake thoroly.
  (4) Allow to stand for several days.
  (5) Distribute in sterile tubes.
  (6) Incubate for 48 hours.

(b) Smith prepared the medium as follows:
  (1) Obtain fresh milk and free from fat by running the milk thru a separator or by allowing the cream to rise spontaneously.
  (2) Test the reaction. It should not be more than +2.
  (3) Tube.
  (4) Sterilize at 100°C. by the fractional method.

A large number of investigators have prepared milk in a manner very similar to that of Smith. Some sterilized the milk in the autoclave. The differences in preparation are not outstanding enough to warrant an individual discussion of each method.

(c) Tanner prepared "Dialyzed Milk" as follows:

(1) Add 0.1 cc. of hydrogen peroxide to 500.0 cc. of milk.
(2) Allow to stand for 2 hours.
(3) Add hydrogen peroxide again (amount not specified).
(4) Allow to stand 4 or 5 hours.
(5) Dialyze (method not given).
(6) Dilute the milk one fifth with water.

References: Reinsch (1892 p. 31), Migula (1901 p. 19), Smith (1902 p. 104), Thoinot and Masselin (1902 p. 27), Committee A.P.H.A. (1905 p. 109), Smith (1905 p. 46), (1905–06 p. 202), Abel (1912 p. 29), Löhnis (1913 p. 20), Tanner (1914 p. 72), Ball (1919 p. 83), Besson (1920 p. 32), Percival (1920 p. 57), Abbott (1921 p. 138), Dopter and Sacquépée (1921 p. 122), Harvey (1921–22 p. 94), Cunningham (1924 p. 16), Park, Williams and Krumwiede (1924 p. 120).

1294. Hiss' Basal Litmus Milk Solution

Constituents:

1. Milk	1000.0 cc.
2. Litmus solution	

Preparation:

(1) Tinge milk with litmus solution.
(2) Dissolve 1.0% of one of the added nutrients in (1).

Sterilization: Method not given.

Use: To study fermentation by dysentery group.

Added nutrients: The author added 1.0% of one of the following:

glucose
maltose
sucrose
dextrin
mannitol

Reference: Hiss (1904–05 p. 31).

1295. Tanner's Azolitmin Milk Powder Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Milk powder, Merrell-Soule	100.0 g.
3. Azolitmus, Kahlbaum's	

Preparation:

(1) Add 100.0 g. of Merrell-Soule's milk powder to 1000.0 cc. distilled water.
(2) Beat with an egg beater.
(3) Add sufficient Kahlbaum's azolitmus to give a color.
(4) Filter.
(5) Distribute in 75.0 cc. quantities.

Sterilization: Method not given.

Use: General culture medium. Green fluorescent bacteria of water, *B. coli*, *B. welchii* and *Bact. acidi lactici* were used.

Variants: Hamilton prepared a similar medium by mixing 1 part purified litmus or azolitmin with 52.7 parts milk powder or 1 part purified azolitmin with 49.6 parts milk flour. One part of this mixture was dissolved in 9.5 parts water, and the medium was sterilized in the autoclave.

References: Tanner (1918 p. 83), Hamilton (1921 pp. 43, 44).

1296. Bacto Litmus Milk (Dehydrated)

Constituents:

1. Distilled water	1000.0 cc.
2. Litmus milk, Bacto	105.0 g.

Preparation:

(1) Dissolve 105.0 g. of Bacto litmus milk (dehydrated) in 1000.0 cc. distilled water.

Sterilization: Sterilize in the usual manner.

Use: Cultivation of organisms causing the souring of milk and dairy products.

Reference: Digestive Ferments Co. (1925 p. 13).

1297. Calandra's Picric Acid Litmus Milk Solution

Constituents:

1. Litmus solution.
2. Picric acid (saturated solution).

Preparation:

(1) Mix 3 parts of a sterile saturated solution of picric acid, 1 part sterile 20.0% NaOH solution and 3 parts sterile litmus tincture solution. Do not heat this mixture.
(2) Add the above sterile indicator mixture to sterile milk.

Sterilization: Sterilize the NaOH, litmus and picric acid separately in the autoclave. Method of sterilization of milk not given.

Use: Differentiation between typhoid bacilli and colon bacilli. Author reported that typhoid bacilli did not cause a change in color nor cause coagulation.

Colon bacilli gave a pea yellow after 24 hours. If a few more drops of picric acid be added to the medium typhoid will not grow while *B. coli* will cause a slight change in color. This indicator may be added to media other than milk.

Reference: Calandra (1910 p. 570).

1298. Park and Krumwiede's Litmus Glycerol Milk Solution

Constituents:

1. Milk........................ 1000.0 cc.
2. Glycerol.................... 50.0 cc.
3. Litmus, 5.0% watery solution Merck's.............. 50.0 cc.

Preparation

(1) Free milk from cream.

(2) Add 5.0% glycerol and 5.0% of a 5.0% watery solution of Merck's purified litmus.

Sterilization: Method not given.

Use: Cultivation of tubercle bacilli. After inoculation seal either with cork alone or preferably dip the cotton stopper also in paraffin.

Reference: Park and Krumwiede (1910 p. 215).

1299. Smith's Litmus Milk Solution

Constituents:

1. Milk.
2. Litmus.

Preparation:

(1) Obtain fresh milk and free from fat by running the milk thru a separator or by allowing the cream to rise spontaneously.

(2) Test the reaction. It should not be more than +2.

(3) Tube.

(4) Add sufficient sterile litmus solution to give a distinct pale blue tinge to the milk, to sterile (3).

Sterilization: Sterilize at 100°C. using the fractional method.

Use: General culture medium.

Variants: Various investigators have prepared media in a number of similar ways. Some investigators used azolitmin instead of litmus, and some sterilized the medium in the autoclave. Others sterilized the medium after the litmus was added. The differences in preparation did not seem to be of sufficient importance to warrant a separate discussion for each investigator.

References: Smith (1902 p. 106), Frost (1903 p. 66), Heinemann (1905 p. 28), Smith (1905 p. 48), Committee A.P.H.A. (1905 p. 109), Roddy (1917 p. 41), Ball (1919 p. 83), Tanner (1919 p. 71), Percival (1920 p. 57), Besson (1920 p. 59), Bezançon (1920 p. 116), Abbott (1921 p. 139), Levine (1921 p. 109), Dopter and Sacquépée (1921 p. 122), Giltner (1921 p. 25), Harvey (1921–22 p. 94), Pitfield (1922 p. 117), Stitt (1923 p. 39), Park, Williams and Krumwiede (1924 p. 120).

1300. Sherman and Albus' Indicator Milk Solution

Constituents:

1. Distilled water............. 1000.0 cc.
2. Milk....................... 1000.0 cc.
3. Methylene blue (medicinal). 0.5 g.

Preparation:

(1) Dissolve 0.5 g. medicinal methylene blue in 1000.0 cc. distilled water.

(2) Add 1.0 cc. of sterile (1) to each 10.0 cc. of sterile milk.

Sterilization: Sterilize the dye and milk separately. Method not given.

Use: To demonstrate the reduction by streptococci of the *Streptococcus lacticus* and *Streptococcus pyogenes* types. The medium was decolorized if reduction occurred.

Variants: The author used the following variants:

(a) Dissolved 1.0 g. of Kahlbaum's indigo carmine in 1000.0 cc. distilled water and used instead of methylene blue solution.

(b) Dissolved 1.0 g. of Grübler's neutral red in 1000.0 cc. of distilled water and used instead of the methylene blue solution.

Reference: Sherman and Albus (1918 p. 167).

1301. Harvey's Brom Cresol Purple Milk Solution

Constituents:

1. Milk........................ 1000.0 cc.
2. Brom cresol purple

Preparation:

(1) Dissolve brom cresol purple in a minimum amount of alcohol, and

make up to 0.04% brom cresol purple by the addition of water.
(2) Add sufficient of (1) to milk to give a distinct color.

Sterilization: Method not specified.

Use: General culture medium. Kahn cultivated spore forming anaerobes.

Variants: Kahn cultivated spore forming anaerobes in a medium prepared as follows:
(1) Add 40.0 cc. of brom cresol purple indicator solution to 1 liter partially skimmed milk (strength of indicator solution not specified).
(2) Tube in 10.0 cc. lots.
(3) Autoclave at 15 pounds for 20 minutes.
(4) The tubes are sealed with a vaseline cap.

References: Harvey (1921–22, p. 88), Kahn (1922, p. 175).

1302. Harvey's China Blue Rosolic Acid Milk Solution

Constituents:

1. Water	100.0 cc.
2. Milk, skimmed	300.0 cc.
3. China blue (1.0% solution)	
4. Rosolic acid (0.5% solution)	

Preparation:
(1) Mix 1 part creamed milk with 3 parts water.
(2) Mix equal parts of a 1.0% watery china blue solution and a 0.5% alcoholic rosolic acid solution.
(3) To each 100.0 cc. of (1) add 2.5 cc. of (2).
(4) Raise to boiling point and boil 5 minutes.
(5) Adjust the reaction by bringing the color, with the addition of alkali, to a pale grey.
(6) Distribute into test tubes containing gas tubes which should project above the surface.

Sterilization: Sterilize in the autoclave.

Use: General culture medium.

Reference: Harvey (1921–22 p. 95).

1303. Bacto Purple Milk, (Dehydrated)

Constituents:

1. Distilled water	1000.0 cc.
2. Purple milk Bacto	105.0 g.

Preparation:
(1) Dissolve 105.0 g. of Bacto Purple Milk (dehydrated) in 1000.0 cc. of distilled water.

Sterilization: Sterilize in the usual manner. The dye will stand heating to a total not over 70 minutes.

Use: General culture medium. Acid producers change the color to yellow, alkali producers to purple.

Reference: Digestive Ferments Co. (1925 p. 13).

1304. Bacto Whey Broth (Dehydrated)

Constituents:

1. Water	1000.0 cc.
2. Whey, Broth, Bacto	30.0 g.

Preparation:
(1) Dissolve 30.0 g. of Bacto Whey Broth (dehydrated) in 1000.0 cc. of distilled water.
(2) If sterilized at 15 pounds pressure for 20 minutes pH = 6.5 ±.

Sterilization: Sterilize in the usual manner.

Use: Cultivation of *Lactobacillus bulgaricus.*

Reference: Digestive Ferments Co. (1925 p. 14).

1305. Stutzer and Hartleb's Whey Solution

Constituents:
1. Whey.

Preparation:
(1) Separate the cream from milk.
(2) Heat to 40°C. and add a little rennet.
(3) After the casein has coagulated heat to 75°C. and filter.
(4) Neutralize by the addition of soda (indicator not specified).

Sterilization: Not specified.

Use: Cultivation of the causitive agent of the foot and mouth disease. Other investigators employed the medium as a general culture medium.

Variants:
(a) The author added Na_2CO_3 to obtain an alkaline reaction, or lactic acid to obtain an acid reaction.
(b) Percival prepared the medium in the following manner:
  (1) Add 10.0 cc. of distilled water containing 8 to 10 drops of commercial rennet extract to 1000.0 cc. of skimmed or separated milk.

(2) Stir briskly for a few seconds.
(3) Allow to coagulate.
(4) When the curd has formed cut with a knife, and leave it for an hour so that the whey may separate.
(5) Filter thru muslin.
(6) Add the white of an egg whipped up with 100.0 g. of water.
(7) Heat in a steam sterilizer for an hour.
(8) Filter into test tubes.
(9) Sterilize on 3 successive days for 20 minutes each day.

(c) Klimmer gave the following method of preparation:
(1) Add a little rennet to milk obtained under the cleanest possible conditions and heat to 40°C. until the casein is coagulated.
(2) Separate the casein from the whey by straining thru a straining cloth.
(3) Filter the whey thru filter paper and then thru an asbestos filter.
(4) Distribute in sterile flasks.
(5) Sterilize by the addition of 1.5% chloroform.

References: Stutzer and Hartleb (1897 p. 403), Percival (1920 p. 58), Klimmer (1923 p. 203).

1306. Emile-Weil's Litmus Whey Solution

Constituents:

1. Whey	1000.0 cc.
2. Litmus	
3. $CaCl_2$	2.0 g.

Preparation:
(1) Skim milk.
(2) Heat to 40°C.
(3) Add an excess of rennet tablets (6 tablets per liter).
(4) Allow to coagulate for 30 minutes.
(5) Cut the curd into large pieces.
(6) Filter thru a thin cloth.
(7) Make the filtrate alkaline to phenolphthalein by the addition of soda.
(8) Add 2.0 g. $CaCl_2$ per liter.
(9) Heat at 110° for 15 minutes.
(10) Filter thru paper until the filtrate is clear.
(11) The reaction should be a deep violet to litmus.
(12) Add 2.0% litmus solution to obtain the desired shade.
(13) Filter thru a candle.
(14) Distribute in test tubes.
(15) Incubate two days in the incubator and 8 days at room temperature to test sterility.

Sterilization: Sterilization is effected in step (13) above.

Use: Cultivation of *Bacillus leprae.* May be used as a general culture medium.

Variants: The author used the medium with a neutral, alkaline or acid reaction.

References: Emile-Weil (1917 p. 380), Besson (1920 p. 59).

1307. Durham's Litmus Whey Solution

Constituents:
1. Whey
2. Litmus

Preparation:
(1) Fresh milk free from antiseptic adulteration is slightly warmed and clotted by means of rennet.
(2) Drain off the whey and hang the clot to drain in a piece of muslin.
(3) Neutralize the turbid or yellow whey with 4.0% citric acid solution, using litmus as an indicator.
(4) Heat upon the water bath for 30 minutes to coagulate the proteins.
(5) Filter and add litmus to obtain a suitable color.
(6) Distribute in 10.0 cc. quantities if desired.

Sterilization: Sterilize at 100°C. If not perfectly clear filter thru a Berkefeld filter after allowing to settle a few days.

Use: Cultivation of the colon-typhoid group.

Variants:

(a) Heinemann prepared the medium as follows:
  (1) Precipitate casein from milk with rennet extract.
  (2) Neutralize the whey with 4.0% citric acid solution.
  (3) Heat on the water bath for 30 minutes.
  (4) Filter.
  (5) Add litmus solution until a decided blue color is obtained.
  (6) Sterilization not specified.

(b) Harvey prepared a similar medium as follows:
  (1) Add rennet to fresh milk.
  (2) Keep at 60°C.

(3) Strain the separated whey thru a thick, clean cloth.
(4) Make the reaction of the fluid neutral to litmus by means of an organic acid, such as citric acid.
(5) Steam 60 minutes.
(6) Filter while hot thru well-wetted, thick filter paper.
(7) Add litmus solution to give a deep purple red color.
(8) Distribute into flasks or test tubes.

(c) Klimmer gave the following method of preparation.
 (1) Coagulate the casein in milk by the addition of rennet by heating at 40°C.
 (2) Filter.
 (3) Boil the filtrate for 2 hours.
 (4) Neutralize.
 (5) Filter.
 (6) The whey should be water clear and slightly yellow.
 (7) Add 5.0 cc. of sterile litmus solution to each 100.0 cc. of whey.
 (8) Add acid or alkali until the medium is a violet color.
 (9) Filter.
 (10) Sterilize (method not given).

References: Durham (1900–01 p. 379), Heinemann (1905 p. 128), Abbott (1921 p. 140), Harvey (1921–22 p. 95), Klimmer (1923 p. 207).

1308. Giltner's Sour Whey

Constituents:
1. Whey.

Preparation:
(1) Inoculate sweet milk with a pure active culture of *Bact. lactis acidi* or *Bact. bulgaricum* as desired and incubate at 30°C.
(2) Allow the maximum acidity to form.
(3) Cut the curd and heat in flowing steam for 20 to 30 minutes.
(4) Strain thru cheese cloth and allow to drain.
(5) Filter thru filter paper.
(6) If clear whey is desired it is necessary to clear the medium by the addition of egg albumin.

Sterilization: Not specified.

Use: To determine the acid destroying ability of bacteria.

Reference: Giltner (1921 p. 365).

1309. Bronfenbrenner, Davis and Morishima's China Blue Rosolic Acid Whey Solution

Constituents:

1. Water	200.0 cc.
2. Whey	100.0 cc.
3. $MnCl_2$ (10.0% soln.)	2.5 cc.
4. China blue, rosolic acid indicator	3.0 cc.

Preparation:
(1) Allow fresh milk to stand and syphon the milk from under the cream.
(2) Bring it to boiling and add 2.5 cubic centimeters of 10% $MnCl_2$ solution to each 100 cubic centimeters of milk.
(3) Cool the mixture as soon as a clot is formed and filter thru a single layer of cloth.
(4) Titrate an aliquot portion hot and adjust the bulk of medium to neutral reaction (1×10^{-7}).
(5) Bring quickly to boiling, cool and filter thru paper.
(6) Dilute the filtrate with double its volume of water and add 1 cubic centimeter of china blue rosolic acid indicator, see medium 535, under variants, for each 100 cubic centimeters of medium. (At this point, the medium will have an intensely blue color).
(7) Distribute into sterile tubes containing inverted fermentation tubes.

Sterilization: Autoclave at 15 pounds pressure for 10 minutes. Immediately after autoclaving the medium will have a pink color. On cooling it will become colorless if it is properly neutralized.

Use: Cultivation of colon-typhoid group. Author reported that the medium gave as good results as lactose-peptone water as a medium for colon-typhoid bacteria and was cheaper and more easily prepared.

Reference: Bronfenbrenner, Davis and Morishima (1918–19 p. 347).

1310. Jouan's Litmus Whey Solution

Constituents:

1. Water	2000.0 or 3000.0 cc.
2. Whey	1000.0 cc.
3. $CaCl_2$ (25.0% soln.)	10.0 cc.

Preparation:

(1) To 1 liter of milk add 10.0 cc. of 15° Baume (equivalent to 25.0 g. crystalline $CaCl_2$ per 100.0 cc. water) $CaCl_2$ solution.
(2) Heat in the autoclave at 115° for 4 or 5 minutes, more or less, depending on the coagulation.
(3) Cool, without shaking.
(4) Filter thru linen.
(5) Add a dilute soda solution until litmus is colored.
(6) Boil for one minute and allow to cool.
(7) Decant and filter. The filtrate is clear.
(8) Mix with 2 or 3 volumes of distilled water.
(9) Add a tincture of litmus solution (amount not given) to give the desired color.
(10) Distribute in tubes or flasks.

Sterilization: Sterilize at 110 to 112°C.

Use: Cultivation of colon typhoid group.

Variants: Besson and Harvey did not dilute the whey with 2 or 3 volumes of water as in step (8) above.

Reference: Jouan (1916 p. 520), Besson (1920 p. 60), Harvey (1921–22 p. 96).

1311. Petruchsky's Litmus Whey Solution (Grimbert and Legros)

Constituents:

1. Whey.
2. Litmus.

Preparation:

(1) Heat cold milk to a gentle heat (temperature not given).
(2) Add sufficient HCl to precipitate the casein.
(3) Filter.
(4) Add soda solution until the reaction is but only slightly acid.
(5) Heat in a Koch steamer for several hours.
(6) Filter.
(7) Neutralize exactly to litmus.
(8) Color with sensitive litmus.

Sterilization: Not specified.

Use: Differentiation of colon-typhoid group. Author reported that colon bacilli turned medium strongly red. Typhoid bacilli produced only slight acidity. *B. faecalis alcaligenes* gave an alkaline reaction.

Variants:

(a) Migula prepared a similar medium as follows:
  (1) Mix equal volumes of fresh milk and water.
  (2) Add HCl until the casein is precipitated.
  (3) Filter.
  (4) Carefully neutralize the whey.
  (5) Boil for 1 to 2 hours.
  (6) Filter and allow to stand until the whey is completely clear.
  (7) The whey may be clarified by the addition of egg white.
  (8) Tube, or distribute in flasks and store until ready for use. Sterilize in steam.
  (9) When ready for use add sufficient litmus tincture (About 5.0 cc. per 100.0 cc. whey) to give a violet color.
  (10) Steam again.
(b) Bezançon gave the following method of preparation for the medium:
  (1) Add drop by drop from a burette 10.0% HCl to 100.0 cc. of boiling milk until the casein is completely coagulated. Stir constantly.
  (2) Add a sufficient quantity of HCl, (calculated from (1)), to cause complete coagulation of casein in 1000.0 cc. of boiling milk. Add the HCl drop by drop, stirring constantly.
  (3) Allow to stand for several hours.
  (4) Decant the liquid whey.
  (5) Filter several times until clear.
  (6) Neutralize.
  (7) Add sufficient litmus solution to give a violet tint.
  (8) Tube.
  (9) Sterilize by heating at 100°C. by the intermittent method.
(c) Besson prepared the medium as follows:
  (1) Dilute 1.0 cc. of HCl in 20.0 cc. of water.
  (2) Add (1) to 1 liter of fresh milk.
  (3) Heat at 110° for 15 minutes.
  (4) Allow to cool.
  (5) Filter thru Chardin paper.
  (6) Make slightly alkaline and heat.
  (7) Add litmus until a light violet color is obtained.

(8) Filter thru a candle.
(9) Distribute.

(d) Harvey prepared the medium as follows:
(1) Add 1.5 cc. strong HCl acid or glacial acetic acid to 1000.0 cc. fresh, slightly warm milk.
(2) Boil.
(3) Filter thru well-wetted, thick filter paper.
(4) Make the reaction of the filtrate neutral to litmus by the addition of dilute sodium carbonate solution.
(5) Filter, while hot thru well-wetted, thick filter paper.
(6) Add litmus solution to give a deep purple red color.
(7) Distribute into test tubes or flasks.
(8) Sterilize.

References: Grimbert and Legros (1901, p. 913), Migula (1901 p. 21), Abel (1912 p. 49), Tanner (1919 p. 71), Bezançon (1920 p. 116), Besson (1920 p. 59), Dopter and Sacquépée (1921 p. 123), Harvey (1921–22 p. 95).

1312. Barsiekow's Basal Nutrose Solution (Segin)

Constituents:

1. Water	1000.0 cc.
2. Nutrose	10.0 g.
3. NaCl	5.0 g.
4. Litmus tincture	100.0 cc.

Preparation:
(1) Dissolve 2 and 3 in 1.
(2) Tube.
(3) Prepare solutions of one of two added nutrients in the litmus tincture.
(4) Add sufficient quantity of sterile (3) to each sterile tube of (2) to give the desired concentration of fermentable material.

Sterilization: Sterilize (2) for an hour in the steamer. Sterilize (3) for 15 minutes in the steamer

Use: To determine fermentation reactions.

Added nutrients and variants:

(a) Segin added one of the following materials:

Lactose
Glucose
Maltose
Erythritol
Dulcitol
Mannitol

(b) Hetsch prepared the medium as follows:
(1) Boil 10.0 g. nutrose, and 5.0 g. NaCl in 1000.0 cc. of distilled water for 2 hours.
(2) Boil 20.0 g. mannitol or 25.0 g. maltose in 50.0 cc. of Kahlbaum's litmus solution for 10 minutes.
(3) Prepare solutions (strength not given) of the sugars and alcohols listed.
(4) Tube in about 10.0 cc. lots and sterilize in flowing steam for ¼ hour.
(5) Add 1 of (4) to (2).

(c) Hiss dissolved 10.0 g. nutrose, 4.0 cc. of normal NaOH 10.0 cc. of 5.0% litmus solution and one of the following in 1000.0 cc. distilled water.

Glucose
Maltose
Sucrose
Dextrin
Mannitol

(d) Elser and Huntoon prepared the medium as follows:
(1) Dissolve 10.0 g. nutrose and 5.0 g. NaCl in 1000.0 cc. distilled water.
(2) Add 5.0 to 7.5 cc. of a watery solution of Merk's highly sensitized litmus.
(3) Sterilize, in usual manner (exact method not specified).
(4) Prepare a 10.0% solution of one of the following in distilled water.

Glucose
Galactose
Levulose
Lactose
Sucrose
Dulcitol
Inulin
Dextrin
Maltose
Mannitol

(5) Sterilize (4) at 100°C. for 10 minutes.
(6) Mix (5) and (3).
(7) Tube in sterile tubes.
(8) Incubate for 3 days to detect accidental contamination.

(e) Tanner prepared the medium as follows:
(1) Dissolve 10.0 g. nutrose and 5.0 g. NaCl in 750 cc. distilled water.
(2) Dissolve 10.0 g. of any desired carbohydrates, alcohol, etc. in 250.0 cc. of water.
(3) Add litmus solution to (2) to give an amethyst color.
(4) Cool (1) and (3).
(5) Mix (1) and (3).

(6) Tube.

(7) Sterilization not specified.

(f) Klimmer gave the following method of preparation:

(1) Mix 10.0 g. of nutrose and 4.0 g. NaCl in a mortar and add water, drop by drop until a thick paste is obtained.

(2) Steam for 1 to 2 hours.

(3) Allow to settle.

(4) Carefully decant and filter thru a folded filter paper.

(5) Dissolve one of the following in 50.0 cc. of Kubel and Tiemann's litmus solution.

lactose	10.0 g.
glucose	10.0 g.
maltose	20.0 g.
sucrose	20.0 g.

(6) Add (5) to (4).

(7) Add 0.1 normal NaOH until the medium is neutral to the litmus.

(8) Distribute in 6.0 cc. quantities in tubes.

(9) Sterilize for 25 to 30 minutes in the steamer.

References: Segin (1903, p. 203), Hetsch (1903, p. 580), Hiss (1904–05, p. 31), Elser and Huntoon (1909, p. 404), Tanner (1919, p. 59), Besson (1920, p. 59), Harvey (1921–22, p. 97), Klimmer (1923, p. 211).

1313. Purwin and McNutt's Basal Nutrose Solution

Constituents:

1. Water	990.0 cc.
2. Nutrose	10.0 g.
3. NaCl	0.5 g.
4. Andrade indicator (1.0%)	

Preparation:

(1) Mix 2 and 3 with 1 and allow to stand over night.

(2) Adjust the reaction to pH 7.7.

(3) Steam in the Arnold for 2 hours.

(4) Filter thru cotton.

(5) Add 1.0% Andrade indicator.

(6) Adjust to a very slight pink if necessary.

(7) Add one of the added nutrients.

Sterilization: Sterilize in the Arnold on each of 3 successive days.

Use: Sugar free broth to study fermentation.

Added nutrients: The authors added any desired carbohydrates.

Reference: Purwin and McNutt (1924 p. 297).

1314. Thöni and Allemann's Nutrose Solution

Constituents:

1. Water	3000.0 cc.
2. Nutrose	30.0 g.

Preparation:

(1) Dissolve 30.0 g. of sodium caseinate (nutrose) in 3 liters of water.

Sterilization: Not specified.

Use: Cultivation of *Bacillus putrificus*. Inoculate and incubate under anaerobic conditions.

Reference: Thöni and Allemann (1916 p. 101).

1315. Ficker and Hoffmann's Caffeine Nutrose Solution

Constituents:

1. Distilled water	200.0 cc.
2. Nutrose	10.0 g.
3. Caffeine	5.0 g.
4. Crystal violet (Höchst)	0.1 g.

Preparation:

(1) Dissolve 10.0 g. nutrose in 200.0 cc. distilled water by boiling on the water bath for several hours.

(2) Make up the loss of water after cooling.

(3) Dissolve 5.0 g. caffeine in 20.0 cc. distilled water at about 80°C. Cool to 55 to 60°C.

(4) Dissolve exactly 0.1 g. Höchst's crystal violet in 100.0 cc. distilled water.

Sterilization: Not specified.

Use: Enrichment of typhoid and cholera bacilli from water and other materials. The author added 900.0 cc. of the water under investigation in a flask. Pour sterile (2) into the flask containing (3). (Do not reverse the order). Mix thoroly. Add this mixture to the water under investigation and add 10.0 cc. of (4). Shake thoroly. Incubate for 12 to 13 hours at 37°C. and then plate on medium. Reitz used the medium for the bacteriological examination of butter. He added 200.0 g. of butter under investigation, melted at 20 or 30°C., to the medium instead of 900.0 cc. of water as above.

References: Ficker and Hoffmann (1904 p. 268), Reitz (1906 p. 722).

1316. Loeffler's Lactose Nutrose Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Nutrose	10.0 g.
3. Lactose	20.0 g.
4. Malachite green (2.0% soln)	50.0 cc.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add 5.0% of a 2.0% malachite green solution to (1).

Sterilization: Not specified.

Use: Enrichment of typhoid bacilli from faeces.

Variants: Klimmer prepared the medium as follows:

(1) Dissolve 10.0 g. nutrose in 1 liter hot distilled water.

(2) Add 20.0 g. lactose to (1).

(3) Flask in 100.0 cc. quantities.

(4) Sterilize on each of 3 successive days for 10 minutes in streaming steam.

(5) Add 5.0 cc. of a 2.0% solution of 120 Höchet malachite green to each 100.0 cc. lot.

References: Loeffler (1906, p. 289–295), Klimmer (1923, p. 213).

1317. Hill's Artificial Milk Medium

Constituents:

1. Distilled water	1000.0 cc.
2. Nutrose	20.4 g.
3. Lactose	10.0 g.

Preparation:

(1) Mix 1, 2 and 3 and allow to stand for 12 hours in the cold.

(2) Shake thoroly and filter thru cotton.

(3) Tube.

Sterilization: Tube and sterilize at 110° for 20 minutes.

Use: Substitute for milk. General culture medium.

Reference: Hill (1909 p. 379).

1318. Doerr's Mannitol Nutrose Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Mannitol	10.0 g.
3. NaCl	5.0 g.
4. Nutrose	10.0 g.
5. Litmus solution, Kahlbaum	30.0 cc.

Preparation:

(1) Add 2, 3 and 4 to 1.

(2) Heat in the water bath until solution is complete.

(3) Filter.

(4) Add 30.0 cc. of Kahlbaum's Litmus Solution.

(5) Distribute into flasks or fermentation tubes.

Sterilization: Sterilize in streaming steam for 15 minutes on two successive days.

Use: Differentiation of colon typhoid and dysentery group. The author reported that Flexner's strains gave red coloration after 24 hours. Shiga group caused no change. Typhoid and coli forms gave the same reaction as in dextrose-nutrose medium, coli causing a precipitation of the nutrose in 24 hours while the typhoid organisms required a longer period to produce precipitation.

Variants: Lehman prepared the medium as follows:

(1) Dissolve 4.0 g. Nutrose (Kahlbaum) and 2.0 g. NaCl in 400.0 cc. distilled water.

(2) Sterilize for 45 minutes in the steamer.

(3) Filter off 300.0 cc. immediately after sterilization.

(4) Dissolve 3.0 g. mannitol in 15.0 cc. of litmus solution by heating slightly.

(5) Mix (4) and (3).

(6) Distribute into 4.0 cc. lots and sterilize for 20 minutes in the steamer.

Reference: Doerr (1903 p. 395), Lehman (1916–17 p. 102).

1319. Whittaker's Lactose Caseinogen Solution

Constituents:

1. Distilled water	1000.0 cc.
2. Caseinogen	15.0 g.
3. Lactose	10.0 g.
4. $CaCl_2$	0.1 g.

Preparation:

(1) Prepare a 1.0% solution of NaOH in distilled water.

(2) Dissolve 15.0 g. of caseinogen in 100.0 cc. of (1). (18 to 24 hours may be required for this.)

(3) Dilute to 900.0 cc. with distilled water.
(4) Add 10.0 g. lactose and 0.1 g. $CaCl_2$ to (3).
(5) Make the solution up to 1000.0 cc. by the addition of distilled water.
(6) Neutralize and make + 0.3 with normal HCl, using phenolphthalein as an indicator.

Sterilization: Sterilize in the autoclave at 107° for 20 minutes.

Use: Substitute for milk. General culture medium.

Reference: Whittaker (1912 p. 162).

1320. Waksman's Basal Casein Solution

Constituents:

1. Water	1000.0 cc.
2. Glycerol	30.0 g.
3. K_2HPO_4	1.0 g.
4. KCl	0.5 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.01 g.
7. Casein	5.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5, 6 and one of the added nutrients in 1.
(2) Dissolve casein in N/10 NaOH and add to (1).
(3) Tube in 10 to 12 cc. lots.

Sterilization: Sterilize at 15 pounds for 15 minutes.

Use: To study metabolism of actinomycetes.

Added nutrients: The author added 2.0 g. of one of the following:

$NaNO_3$	$(NH_4)_2SO_4$
$NaNO_2$	$(NH_4)_2CO_3$

Reference: Waksman (1920 p. 3).

1321. Seliber's Basal Casein Solution (Harvey)

Same as medium 743 but not containing peptone.

1322. Laxa's Lactic Acid Casein Solution

Constituents:

1. Water	1000.0 cc.
2. NaCl	5.0 g.
3. $CaCl_2$	0.1 g.
4. $MgCl_2$	0.2 g.
5. Potassium phosphate (neutral)	2.5 g.
6. Lactic acid	3.0 g.
7. Casein	30.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Suspend 30.0 g. of casein in (1).

Sterilization: Method not given.

Use: Cultivation of oidium.

Reference: Laxa (1901–02 p. 129).

1323. Baginsky's Casein Solution

Constituents:

1. Water	1000.0 cc.
2. Casein	1000.0 g.

Preparation:
(1) Add 20.0 g. of casein to 20.0 cc. of water (or nutrient solution, composition not given).
(2) Distribute into sterile flasks.

Sterilization: Sterilize in the steamer.

Use: To study fermentation by *B. lactis*.

Reference: Baginsky (1888 pp. 434–462).

1324. Harvey's Alkaline Casein Solution

Constituents:

1. Water	1000.0 cc.
2. Casein	20.0 g.
3. NaOH (normal)	10.0 cc.

Preparation:
(1) Mix 1, 2 and 3.

Sterilization: Not specified.

Use: Cultivation of anaerobic organisms. Author specified that nutrose or any other casein product could be used instead of casein.

Reference: Harvey (1921–22 p. 96).

1325. Geilinger's Basal Urine Solution

Constituents:
1. Urine, cow.

Preparation:
(1) Mix one of the added nutrients with sterile cow's urine.

Sterilization: Sterilize cow's urine by heating for one hour at 0.5 atmosphere pressure or preferably by filtering thru a Chamberland clay candle filter.

Use: Cultivation of organisms capable of splitting urea, *Bac. urea*.

Added nutrients and variants:
(a) The author added 1.0 cc. of one of the following to each 20.0 cc. of sterile urine.
The added nutrients were prepared as follows:
  (a) Straw infusion.
    (1) Mix 9 parts by weight of water with 1 part by weight

of finely chopped straw and heat in steamer for one hour.
   (2) Filter.
   (3) Sterilize for 45 minutes at 0.5 atmosphere pressure.
  (b) Feces infusion.
   (1) Mix one part cow feces with 4 parts water.
   (2) Heat for one hour in the steamer.
   (3) Filter.
   (4) Sterilize for 45 minutes under 0.5 atmosphere pressure.
  (c) Peptone solution.
   (1) Prepare and filter a 10.0% peptone solution.
   (2) Sterilize (method not given).
 (b) The author used cow's urine without any additions.

Reference: Geilinger (1917 p. 246).

1326. Bokorny's Sucrose Urine Solution

Constituents:

1. Urine	1000.0 cc.
2. Sucrose	80.0 g.

Preparation:
 (1) Add 80.0 g. sucrose to 1000.0 cc. of undiluted and not neutralized urine.

Sterilization: Not specified.

Use: To study the growth of yeast. The author reported that neutralized urine showed better growth than non-neutralized. The effect of diluting the urine was that of diluting the food materials. Growth best if diluted urine be neutralized.

Variants: The author added 80.0 g. of sucrose to 1000.0 cc. of urine prepared in the following manner:
 (a) Undiluted urine neutralized with K_2HPO_4.
 (b) Undiluted urine partially neutralized.
 (c) Unneutralized urine mixed with an equal volume of water.
 (d) Neutralize urine with K_2HPO_4 and then add an equal amount of water.
 (e) Neutralize urine with K_2HPO_4 and dilute with 3 volumes of water.
 (f) Dilute unneutralized urine with 5 times water.
 (g) Neutralize urine with K_2HPO_4 and dilute 5 times with water.

Reference: Bokorny (1920 p. 27).

1327. Besson's Urine Medium (Tanner)

Constituents:
 1. Urine.

Preparation:
 (1) Boil some recently passed urine.
 (2) If the reaction is markedly alkaline after boiling, add a little tartaric acid solution. Use litmus paper as an indicator.
 (3) Filter.
 (4) Tube.

Sterilization: Sterilize at 155°C. It is better to filter thru a Chamberland bougie.

Use: General culture medium.

Variants: The following variants have been given by the following authors:
 (a) Besson—
  (1) Collect urine under aseptic conditions in sterile flasks.
  (2) Distribute in tubes.
  (3) Incubate for 24 hours at 37°C. to test sterility.
 (b) Besson filtered fresh urine thru a Chamberland filter.
 (c) Besson—
  (1) Boil fresh urine.
  (2) Neutralize to litmus by the addition of tartaric acid if necessary.
  (3) Filter.
  (4) Tube.
  (5) Sterilize at 115°C.
 (d) Harvey—
  (1) Collect urine fresh.
  (2) Boil.
  (3) Filter.
  (4) Sterilize in the steamer or autoclave.
 (e) Harvey—
  (1) Distribute into test tubes freshly passed urine diluted to specific gravity 1.010.
  (2) Sterilize in the steamer or autoclave.

References: Tanner (1919 p. 58), Besson (1920 p. 33), Harvey (1921–22 p. 84).

1328. Burri and Stutzer's Nitrate Feces Solution

Constituents:

1. Water	1000.0 cc.

2. Feces (horse)	50.0 g.
3. $NaNO_3$	3.2 g.

Preparation:
(1) Mix 1, 2 and 3.

Sterilization: Do not sterilize.

Use: Enrichment of nitrate reducers, and to study denitrification.

Variants:
(a) Ampola and Garino specified cow feces instead of horse.
(b) Jensen mixed 10.0 g. $NaNO_3$, 100.0 g. cow or horse feces and 0.0 or 20.0 cc. of glycerol. He reported that the presence of glycerol aided denitrification.

References: Burri and Stutzer (1895 p. 261), Ampola and Garino (1896 p. 671), Jensen (1897 p. 693).

1329. Dimitroff's Fecal Infusion

Constituents:

1. Tap water	1000.0 cc.
2. Feces (human) (1.0 to 2.0%)	10.0 to 20.0 g.

Preparation:
(1) Prepare an emulsion of 1.0 to 2.0% human feces in tap water.

Sterilization: Method not given.

Use: Cultivation of *Leptospira biflexa.*

Reference: Dimitroff (1927 p. 511).

1330. Dunham's Meat Infusion Solution

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.

Preparation:
(1) Boil finely chopped beef with a double weight of water for 2 hours.
(2) Filter.
(3) Make slightly alkaline; 0.5% NaCl may be added if desired.

Sterilization: Not specified.

Use: Dunham used the solution for the detection of cholera bacilli. He reported that the cholera vibrio gave a red ring where the H_2SO_4 and medium met when concentrated H_2SO_4 was poured down the side of the tube containing a culture. Similar media were used by different investigators for a variety of purposes.

Variants: The following investigators prepared media as follows:
(a) Migula—
(1) Mix 500.0 g. of finely chopped lean beef with one liter of water, and allow to stand in the ice box for 12 to 24 hours.
(2) Press the liquid thru a towel and make up the volume to one liter.
(3) Boil in the steam cooker for 30 minutes.
(4) The infusion may be boiled for an hour before removing the meat, and then filtered thru paper. If the liquid is still red, boil again for 15 minutes.
(5) Distribute in flasks.
(6) Boil for an hour to sterilize.
(7) Store until ready for use. Seal the flask with paraffin, gum arabic or a beaker if it is to be stored for a long time.
(b) Abel—
(1) Chop 500.0 g. of fat-free meat and add to a liter of water at 50°C.
(2) Keep at 50°C. for 30 minutes and then boil for 30 to 45 minutes.
(3) Filter or strain the fluid from the meat.
(4) Make up the fluid to one liter.
(5) Sterilize on each of 3 successive days in the steamer, or autoclave, for 15 minutes at 120°C.
(c) Linde—
(1) Chop 500.0 g. of beef into small pieces.
(2) Boil with one liter of water.
(3) Filter.
(4) Use filtrate without any additions or add a concentrated Na_2CO_3 solution to give alkaline reaction.
(5) Sterilization not specified.
(d) Davis and Ferry used beef infusion (or a 1.0% meat extract solution) as a basic solution and added one of the following to 1000.0 cc. of the basic solution:

Cystine	0.5 g.
Tryptophane	0.6 g.
Tyrosine	1.25 g.
Glutaminic acid hydrochloride	2.5 g.
Histidine dichloride	0.5 g.
Leucine	3.0 g.
Glycocoll	0.75 g.
Sodium asparaginate	1.5 g.
Glucoseamine hydrochloride	2.0 g.
Creatine	0.2 g.
Creatinine	0.15 g.

Xanthin	0.05 g.
Hypoxanthin	0.05 g.
NaCl	4.0 g.
K_2HPO_4	3.0 g.
$MgSO_4$	0.4 g.
KNO_3	0.2 g.

These media were used for the cultivation of *Bact. diphtheriae* and toxin production by it.

(e) Waksman studied the metabolism of actinomyces in media prepared as follows:

(1) Boil beef in water, filter and sterilize.

(2) Inoculate with *B. coli communor*, incubate at 37° for 24 hours.

(3) Boil for 10 minutes, adjust to pH = 7.6 to 7.7. Boil again, filter and sterilize.

(4) Add 1.0% of any desired carbohydrate, alcohol, etc., and 1.0% of Andrade indicator.

(5) Distribute in fermentation tubes.

(6) Steam in Arnold for 30 minutes to sterilize.

The author reported that the actinomycetes developed in the infusion alone without the addition of other nutrients.

(f) Besson—

(1) Pour 1000.0 cc. of water on 500.0 g. of finely chopped beef, and place in the ice box for 12 hours.

(2) Stir the mixture well.

(3) Filter thru a cloth and press the meat free from juice.

(4) Filter the juice thru paper.

(5) Add 5.0 g. NaCl.

(6) Boil.

(7) Make slightly alkaline or neutralize to litmus by the addition of soda.

(8) Heat at 115 to 117°C. for 5 minutes.

(9) Filter until clear.

(10) Make up to 1000.0 cc. by the addition of distilled water.

(11) Distribute as desired.

(12) Sterilize at 110 to 115°C. for 20 minutes.

(g) Giltner—

(1) Add 500.0 g. of tap water to 500.0 g. finely chopped fresh lean beef in 3.5 liter agate ware pail.

(2) Mix thoroly and allow to stand in a cool place (refrigerator) for not more than 16 to 24 hours.

(3) Strain the infusion thru a cheese cloth, thoroly pressing out all the juice.

(4) Make up to 500.0 cc. by the addition of tap water if necessary.

(5) Place (4) in a sterile liter Erlenmeyer flask.

(6) Heat in the autoclave at 120°C. for 30 minutes.

(h) Harvey—

(1) Mince finely fat-free beef.

NOTE: Veal, chicken, ox or horse heart, horse flesh, rabbit flesh, fish, blood, placenta, liver, spleen, kidneys, brain and vegetable materials, such as yeast, wheat, etc., may serve to furnish the extract used as basis for the medium.

(2) Add 500.0 g. to 1000.0 cc. distilled water or clear tap water.

(3) Heat the mixture 20 minutes over a free flame, at a temperature not exceeding 50°C.

NOTE: Or simply keep in a cool place over night.

(4) Skim off fat floating on the surface.

(5) Raise the temperature to boiling point.

(6) Boil 10 minutes.

(7) Pour the mixture on to a wet, thick, clean cloth.

(8) Add sodium chloride 5.0 g. to the filtrate.

(9) Steam 45 minutes.

(10) Bring the volume up to 1000.0 cc. by the addition of water.

(11) Estimate and adjust the reaction.

(12) Steam 30 minutes.

(13) Filter, while hot, thru well-wetted, thick filter paper.

References: Dunham (1887 p. 338), Migula (1901 p. 13), Abel (1912 p. 13), Linde (1913 p. 386), Davis and Ferry (1919 p. 235), Waksman (1919 p. 316), Besson (1920 p. 28), Dopter and Sacquépeé (1921 p. 120), Giltner (1921 p. 27), Harvey (1921–22 p. 94).

1331. Stutzer and Hartleb's Nitrite Infusion Solution

Constituents:

1. Water........................ 2000.0 cc

2. Meat	1000.0 g.
3. $NaNO_2$ (2.0%)	40.0 g.

Preparation:

(1) Add 2 liters of water to 1000.0 g. of finely chopped lean meat.
(2) Heat in the steamer at 100°C. for 2 hours.
(3) Pour off the liquid.
(4) Make (3) up to 2 liters.
(5) Neutralize by the addition of soda, indicator not specified.
(6) Add 2.0% $NaNO_2$.
(7) Filter.

Sterilization: Method not given.

Use: Cultivation of bacteria from case of foot and mouth disease.

Reference: Stutzer and Hartleb (1897 p. 404).

1332. Besson's Glucose Infusion Solution

Constituents:

1. Beef infusion solution	1000.0 cc.
2. Glucose	4.0 g.
3. Neutral red (1.0% soln.)	3.0 cc.

Preparation:

(1) Method of preparation of beef infusion solution not given.
(2) Dissolve 2 in (1).
(3) Add 3.0 cc. of a 1.0% watery neutral red solution.
(4) Distribute in tubes containing fermentation tubes.

Sterilization: Sterilize at 110°C., time not specified.

Use: Differentiation of colon typhoid group. Author reported that *Bact. typhosum* and dysentery gave amaranth tint, but no gas. Paratyphoid A, amaranth tint with a gas bubble. Paratyphoid B gave orange color in the tube with yellow in the fermentation tube, gas was produced. *B. proteus* same as paratyphoid B but much less gas was produced. Colon bacilli, fluorescence with a yellow tint, gas was produced. *B. fecal alcaligenes,* no fluorescence. Culture salmon colored in the tube, gas was not produced.

Reference: Besson (1918 p. 929).

1333. Harvey's Starch Beef Infusion

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.
3. NaCl	5.0 g.
4. Starch	10.0 g.

Preparation:

(1) Prepare meat infusion solution from 1, 2 and 3 as indicated in medium 850.
(2) Add 10.0 g. starch to 1000.0 cc. of (1).
(3) Adjust to 0.4% to phenolphthalein.

Sterilization: Not specified.

Use: Cultivation of gonococci.

Reference: Harvey (1921–22 p. 112).

1334. Beijerinck's Urea Meat Infusion

Constituents:

1. Meat infusion	1000.0 cc.
2. Urea (4.0%)	40.0 g.

Preparation:

(1) Add 4.0% urea to meat infusion.

Sterilization: Not specified.

Use: Cultivation of *Urococcus ureae, Urobacillus pasteurii, Urobacillus leubei, Planosarcina ureae* and other bacteria.

Variants:

(a) The author used 5.0, 6.0, or 10.0% urea instead of 4.0%.
(b) Söhngen added 0.5 to 6.0% urea to meat infusion or bouillon to cultivate *Bacillus erythrogenes*.
(c) Groenewege cultivated *Phytobacter lycopersicum,* (causing tomato rot) in meat infusion solution containing 0.5% urea.
(d) Wojtkiewicz studied urea decomposition by soil forms in a meat infusion solution containing 0.5% NaCl and 10.0% urea.

References: Beijerinck (1901 p. 52), Söhngen (1909 p. 95), Groenewege (1913 p. 24), Wojtkiewicz (1914 p. 259).

1335. Klimmer's Blood Infusion

Constituents:

1. Water	1000.0 cc.
2. Blood	500.0 cc.

Preparation:

(1) Mix blood with a double amount of water. The clot of the blood is previously run thru a meat chopping machine.
(2) Boil for 10 minutes, stirring continuously to prevent burning.
(3) Acidify slightly by the addition of acetic acid.
(4) Boil for 5 more minutes.
(5) Allow to settle and cool.

(6) Decant carefully and strain thru a straining cloth.
(7) Filter thru paper.

Sterilization: Not specified.

Use: Use as meat water in the preparation of media.

Reference: Klimmer (1923 p. 172).

1336. Szasz's Blood Clot Infusion

Constituents:

1. Distilled water	2000.0 cc.
2. Blood clot	1000.0 g.

Preparation:

(1) Take 1000.0 g. of blood from which the serum has been separated, coagulated blood or blood clots and add 2000.0 cc. of distilled water.
(2) Boil for a short time. (Time not specified.)
(3) Separate the clot into pieces the size of a nut and boil longer. Do not cut the clot before heating. Take care that the blood clot does not sink to the bottom of the kettle and burn. Place the kettle or container in which the boiling is taking place over a free flame. It may be stirred with a wooden spoon. A linen towel may be placed in the bottom of the container and extend part way up the side walls or all the way up and be fastened with a cord. This is the simplest way to prevent burning.
(4) Filter thru a large linen towel.
(5) The bouillon may be clarified with egg white.

Sterilization: Not specified.

Use: Inexpensive medium. Meat infusion or extract substitute.

Variants:

(a) The author prepared a similar medium as follows:
  (1) Divide the blood clots into pieces the size of a walnut or hazel nut by means of a piece of wood or hands.
  (2) To the clot obtained from every kilogram of blood, add 1.5 liters of water.
  (3) Mix well and allow to stand in the cold for 20 to 24 hours, stirring several times.
  (4) Filter thru a coarse linen cloth and boil the filtrate until brown clumps are formed and the liquid is yellow.
  (5) Make up to the original volume, adjust the reaction as desired in the same manner as for meat extract.
  (6) The bouillon may be filtered until clear.
  (7) Usually 0.5% glycerol is added.
  (8) Sterilization not specified.

(b) Stefanopoulo cultivated *Spirochaeta icterohemorragiae* on a medium prepared as follows:
  (1) Separate the serum from the clot of coagulated horse blood.
  (2) Press the clot thru a fine wire gauze.
  (3) Dilute with two times its volume with physiological salt solution prepared by dissolving 8.0 g. of NaCl in 1000.0 cc. of water.
  (4) Heat for 15 minutes at 80°C.
  (5) Filter thru paper, and then on a Chamberland filter.
  (6) Distribute in sterile tubes and cover with a layer of sterile vaseline.

References: Szasz (1914–15 p. 491), (1915–16 p. 111), Stefanopoulo (1921 p. 813).

1337. Wellman's Placenta Infusion

Constituents:

1. Distilled water	1000.0 cc.
2. Placenta, human	1000.0 g.

Preparation:

(1) Grind fresh human placenta thoroly in a meat chopping machine after first washing out the blood by running sterile salt solution thru the attached vessel.
(2) To each kilogram of macerated placental tissue add 1 liter of distilled water.
(3) Infuse for 48 hours in the ice box.
(4) Tube sterile (3).
(5) Store the medium at 40–41°C. for 2 days before use to inactivate the complement.

Sterilization: Pass thru a No. N Berkefeld filter that will hold back ordinary bacteria. To facilitate this fill the cylinder of the filter with a clean fine sterile sand until the cylinder is completely covered.

Use: Cultivation of parasitic bacteria. Author used *Bacillus leprae* and tubercle bacilli.

Variants:

(a) Gröer and Srnka studied the production of toxin by diphtheria bacilli, and cultivated them on a medium prepared as follows:

(1) Mix 4 liters of medium fine placenta with 4 liters of water.
(2) Boil for about 3 hours with the addition of water.
(3) Filter.
(4) Concentrate the filtrate to 3 liters.
(5) Add N/1 10.0% NaOH until phenolphthalein is turned weakly red. (Hot titration).
(6) Distribute into flasks and sterilize. (Method not given).

(b) Park, Williams and Krumwiede prepared a similar medium as follows:

(1) Add 500.0 g. of ground up tissue (placenta) to a liter of water.
(2) Soak for 90 minutes.
(3) Strain thru cheese cloth and squeeze by twisting the cloth or use a meat press.
(4) Filter thru paper or sand.
(5) Sterilize by filtration thru a filter candle.

References: Wellman (1912 p. 143), Gröer and Srnka (1918–19 p. 334), Park, Williams and Krumwiede (1924 p. 125).

1338. Moon's Brain Infusion

Constituents:

1. Physiological salt solution... 900.0 cc.
2. Brain (dog)................. 100.0 g.

Preparation:

(1) Remove brain matter from normal dog under the most careful aseptic precautions.
(2) Add 900.0 cc. of physiological salt solution to 100.0 g. of (1).
(3) Emulsify by shaking with glass beads.

Sterilization: Filter thru a coarse Berkefeld filter.

Use: Cultivation of Negri bodies. Author reported that growth was poor, both anaerobically and aerobically.

Reference: Moon (1913 p. 233).

1339. Wolbach and Saiki's Liver Infusion

Constituents:

1. Distilled water............. 1000.0 cc.
2. Liver (dog)................ 1000.0 g.

Preparation:

(1) Pass fresh dog's liver thru the meat grinder.
(2) Mix equal parts of (1) and distilled water.
(3) Shake vigorously.
(4) Filter thru Buchner filters and then thru Berkefeld filters (finest grade).
(5) Distribute to fermentation tubes.
(6) Incubate for 24 hours to determine sterility.

Sterilization: Sterilization given under preparation.

Use: Cultivation of a spore bearing anaerobe from a dog's liver. The medium obtained at first is rather viscous, red and slightly acid in reaction. After 48 hours incubation proteins are precipitated.

Reference: Wolbach and Saiki (1909 p. 270).

1340. Brieger's Thymus Gland Infusion (Besson)

Constituents:

1. Distiled water.............. 1000.0 cc.
2. Thymus gland (beef)....... 1000.0 g.

Preparation:

(1) Remove the thymus glands from dead beef.
(2) Chop into a pulp and add an equal weight of distilled water.
(3) Allow to soak for 12 hours.
(4) Filter thru a gauze, pressing out the liquid.
(5) Add an equal weight of water to the turbid viscous liquid.
(6) Make slightly alkaline by the addition of a 1.0% solution of sodium bicarbonate.
(7) Heat at 100°C. for 15 minutes in the autoclave or salt bath.
(8) Filter thru a fine linen cloth.
(9) Distribute in sterile tubes.

Sterilization: Sterilize at 100° for 15 minutes on two days.

Use: General culture medium. Besson reported that the cholera vibrio did not develop in this medium unless it was diluted with 5 or 6 volumes of sterile water before use.

Reference: Besson (1920 p. 30).

1341. Nencki, Sieber and Wyznikiewicz's Salivary Gland Infusion

Constituents:

1. Distilled water............. 1000.0 cc.
2. Salivary glands............ 200.0 g.

Preparation:
- (1) Obtain beef salivary glands from the slaughter house.
- (2) Cut in small pieces with a meat chopper.
- (3) Add 5 volumes of water.
- (4) Filter thru blotting paper.
- (5) Distribute sterile (4) into sterile tubes.

Sterilization: Filter thru a Chamberland filter to sterilize.

Use: Medium when inoculated with plague containing material contained round cells after two days.

Variants:
- (a) The author added 3.0% NaCl or 2.0 to 3.0% NaCl plus 0.2 to 0.5 g. NaOH or KOH per liter.
- (b) Mayer prepared a similar medium as follows:
  - (1) Chop fresh salivary glands in a meat chopping machine.
  - (2) Mix with an equal weight of water.
  - (3) Infuse on ice for 24 hours after stirring strongly.
  - (4) Press the mass in a meat press.
  - (5) Sterilize the thick, slimy liquid in streaming steam for 30 minutes. Mayer reported that a meat extract medium generally gave better growth of parasitic and saprophytic bacteria.

References: Nencki, Sieber and Wyznikiewicz (1898 p. 530), Mayer (1899 p. 747).

1342. Graham-Smith's Heart Infusion

Constituents:

1. Distilled water	250.0 cc.
2. Heart, bullock	100.0 g.

Preparation:
- (1) Remove the fat and vessels from fresh bullock's heart muscle.
- (2) Pass thru a mincing machine.
- (3) To each 100.0 g. of (2) add 250.0 cc. of water.
- (4) Boil gently for 90 minutes.
- (5) Filter.

Sterilization: Sterilize for 20 minutes on 3 successive days in a steam sterilizer.

Use: General culture medium. Robertson and Davis used a similar medium to study the influence of vitamines on bacterial growth. They reported that the medium did not give continued growth of yeast.

Variants: Robertson and Davis prepared a similar medium in the following manner:
- (1) Grind lean beef heart finely and just cover with sterile distilled water.
- (2) Place in ice box 18 hours.
- (3) Pass thru Berkefeld filter.
- (4) Mix (3) with sterile (method of sterilization not given) physiological salt solution.

Reference: Graham-Smith (1920–21 p. 136), Robertson and Davis (1923 p. 154).

1343. Robertson and Davis' Heart Infusion Medium

Constituents:

1. Water, sterile distilled	1000.0 cc.
2. Asparagin (Merck)	3.4 g.
3. $CaCl_2$	0.1 g.
4. Glucose	20.0 g.
5. $MgSO_4$	0.2 g.
6. K_2HPO_4	1.0 g.
7. NaCl	5.0 g.
8. Heart, beef.	

Preparation:
- (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1 by boiling 3 minutes.
- (2) Restore original volume with sterile distilled water.
- (3) Adjust reaction to $pH = 7.4$ and tube.
- (4) Grind lean beef heart finely and just cover with sterile distilled water.
- (5) Place in ice box 18 hours.
- (6) Pass thru Berkefeled filter.
- (7) Add various amounts of (6) to each tube of sterile (3).

Sterilization: Autoclave (3) at 20 pounds pressure for 30 minutes. Heart infusion is sterilized by filtration thru Berkefeld filter.

Use: To study influence of vitamins on bacterial growth. Author reported a luxuriant growth of yeast in this medium.

Reference: Robertson and Davis (1923 p. 154).

1344. Mereshkowsky's Egg White Infusion

Constituents:

1. Water	1000.0 cc.
2. Egg albumin	100.0 g.

Preparation:
- (1) Remove the shell from hard boiled hen eggs.
- (2) Cut the egg in 4 lengthwise strips and remove the egg yolk.

(3) Pass the egg white thru a meat grinding machine.
(4) To each 100.0 g. of finely chopped egg white add 1000.0 cc. of water.
(5) Place in a flask and plug.
(6) Autoclave for 15 minutes under one atmosphere of pressure.
(7) Filter thru cotton, and then thru paper.
(8) Distribute into test tubes.

Sterilization: Method not given.

Use: Cultivation of *Bacillus Danysz.* The author reported that *Bacillus Danysz* did not lose its virulence on repeated cultivation on this medium.

Reference: Mershkowsky (1912 p. 394).

1345. Proca's Spleen Infusion Solution

Constituents:

1. Ringer solution	1000.0 cc.
2. Spleen	400.0 g.

Preparation:

(1) Add 400.0 g. of finely chopped fresh beef spleen to 1000.0 cc. of Ringer's solution (see medium #180).
(2) Heat at 115°C. for 30 minutes.
(3) Filter while hot thru paper.
(4) Distribute in tubes or flasks. (The filtrate may be solidfied with 1.4% agar or 10.0% gelatin).

Sterilization: Sterilize in the autoclave.

Use: General culture medium.

Reference: Proca (1924 p. 1164).

1346. Harde and Hauser's Fish Infusion

Constituents:

1. Water	1000.0 cc.
2. Fish (Whiting)	500.0 g.

Preparation:

(1) Boil 500.0 g. of chopped whiting fish with 1 liter of water for 20 minutes.
(2) Filter thru paper.
(3) Reaction is neutral to litmus.

Sterilization: Sterilize at 120° for 20 minutes.

Use: Substitute for beef media and as a general culture medium.

Reference: Harde and Hauser (1919 p. 1259).

1347. Pergola's Mussel Infusion

Constituents:

1. Water	1000.0 cc.
2. Mussels	500.0 g.

Preparation:

(1) Remove mussels from their shells.
(2) Work (1) into a pulp and mix with a double weight of water.
(3) Place in the autoclave for 15 to 20 minutes under 1 atmosphere pressure.
(4) Filter thru filter paper until a not exccessive turbid, at the most a slightly opalescent, liquid is obtained.
(5) Place in the autoclave for 30 minutes again under 1 atmosphere pressure.
(6) Filter. The filtrate is a clear amber yellow.
(7) Distribute into sterile tubes.

Sterilization: The medium may be sterilized in the autoclave if necessary.

Use: Cultivation of typhoid, cholera and other intestinal forms. Author reported that the cholera vibrio lost its characteristic appearance on this medium.

Variants:

(a) The author used oysters instead of mussels.
(b) The medium was prepared as follows:
  (1) Remove oysters or mussels from their shell.
  (2) Work (1) into a pulp and mix with a double weight of sterile phyiological salt solution.
  (3) Allow the mixture to stand for 24 to 48 hours at 15°C.
  (4) Pour off the liquid, filter thru cotton then thru paper and finally thru a Berkefeld candle.
  (5) This gives a yellowish amber fluid. The author reported that the organisms failed to develop on this medium.

Reference: Pergola (1912 p. 171).

1348. Besson's Chicken Infusion (Tanner)

Constituents:

1. Water	1000.0 cc.
2. Chicken	500.0 g.

Preparation:

(1) Soak 500.0 g. of lean chicken meat in 1000.0 cc. water.
(2) Filter.

Sterilization: Not specified.

Use: Substitute for beef infusion.

Reference: Tanner (1917 p. 46).

1349. Büsgen and Höflich's Meat Extract Solution (Linde)

Constituents:

1. Water	1000.0 cc.
2. Meat extract (0.5%)	5.0 g.

Preparation:
(1) Dissolve 2 in 1.

Sterilization: Not specified.

Use: Enrichment of Cladothrix, *Cladothrix dichotoma*. Cultivation of other organisms.

Variants:
(a) The author added a concentrated Na_2CO_3 solution to obtain an alkaline reaction.
(b) Zikes cultivated cladothrix and *Sphaerotilus natans* in a medium containing 0.5, 0.25, 0.125, 0.063 or 0.031% meat extract.
(c) Besson prepared the medium as follows:
  (1) Dissolve 0.5% Liebig's meat extract (or 20.0 g. Cibils meat extract) in 1000.0 cc. of water.
  (2) Make alkaline if necessary.
  (3) Autoclave at 115 to 117° for 5 minutes.
  (4) Filter while hot thru a wet filter.
  (5) Tube.
  (6) Sterilize at 110 to 115°C.

Reference: Linde (1913 p. 372), Zikes (1915 p. 530), Besson (1920 p. 30).

1350. Stutzer's Nitrate Meat Extract Solution

Constituents:
1. Water
2. KNO_3
3. Liebig's Meat Extract

Preparation:
(1) Dissolve 2 and 3 in 1.
(2) Adjust the reaction to a slight alkalinity.
(3) Distribute in 10.0 cc. lots.

Sterilization: Method not given.

Use: To study denitrification by *B. agilis*, *B. nitrovorus*, *B. Stutzeri*, *B. Hartlebi*.

Variants:
(a) The author used Schülke and Meyer or Cibils meat extract instead of Liebig's.
(b) Tanner dissolved 10.0 g. c.p. $NaNO_3$ and 3.0 g. of Liebig's meat extract in 1000.0 cc. of water.

Reference: Stutzer (1901 p. 83), Tanner (1919 p. 45).

1351. Zikes' Basal Glucose Meat Extract Solution

Constituents:

1. Water	1000.0 cc.
2. Meat extract (0.5%)	5.0 g.
3. Glucose (0.25%)	2.5 g.

Preparation:
(1) Dissolve 2, 3 and one of the added nutrients in 1.
(2) Distribute as desired.

Sterilization: Not specified.

Use: Author used the medium to study the nitrogen sources suited to the development of *Cladothrix dichotoma* and *Cladothrix natans*. He reported that *Cladothrix dichotoma* grew well on any nitrogen source but *Cladothrix natans* grew only when peptone or asparagin was added. Blöch studied the nitrogen sources suited for the development of *Zoogloea ramigera*. He reported that asparagin peptone, ammonium sulphate and then nitrate was the order of the materials as a nitrogen source for *Zoogloea ramigera*.

Added nutrients: Zikes as well as Blöch added 0.25% of one of the following:

$(NH_4)_2SO_4$	asparagin
KNO_3	peptone

Reference: Zikes (1915 p. 542), Blöch (1918 p. 51).

1352. Stutzer's Glucose Meat Extract Solution

Constituents:
1. Water.
2. Glucose.
3. KNO_3
4. Liebig's Meat Extract.

Preparation:
(1) Dissolve 2, 3 and 4 in 1. (Peptone may be added).
(2) Reaction to be slightly alkaline.
(3) Distribute in 10.0 cc. lots.

Sterilization: Method not given.

Use: To study denitrification by *B. agilis*, *B. nitrovorus*, *B. Stutzeri*, *B. Hartlebi*.

Variants:
(a) The author substituted Schülke and Meyer's meat extract or Cibils meat extract for Liebig's.

(b) Kappen studied nitrate decomposition by cyanamide decomposing bacteria, in a medium composed of 5.0 g. meat extract, 2.0 g. glucose and 2.0 g. KNO_3 per liter.

Reference: Stutzer (1901 p. 83), Kappen (1909 p. 395).

1353. Hurler's Succinate Meat Extract Solution

Constituents:

1. Water	1000.0 cc.
2. Meat extract, Cibils	100.0 to 200.0 cc.
3. Ammonium succinate	10.0 g.

Preparation:

(1) Thicken 100 to 200.0 cc. of Cibils meat extract on the water bath.

(2) Transfer the viscous mass to a crucible and carbonize and ash under the hood.

(3) Take 3.0 g. of the grey white ash thus obtained and dissolve in 1 liter of water by boiling an hour.

(4) Filter repeatedly thru cotton.

(5) Add 10.0 g. of ammonium succinate and neutralize with dilute ammonia.

(6) Distribute the slightly opalescent medium in tubes.

Sterilization: Sterilize (method not given). The medium becomes clear.

Use: Cultivation of typhoid and intermediate group.

Reference: Hurler (1912 p. 356).

1354. Blöch's Basal Asparagin Meat Extract Solution

Constituents:

1. Water	1000.0 cc.
2. Meat extract (1.0%)	10.0 g.
3. Asparagin (0.25%)	2.5 g.

Preparation:

(1) Dissolve 2, 3 and 0.25% of one of the added nutrients in 1.

(2) Tube or flask.

Sterilization: Not specified.

Use: Cultivation of *Zoogloea ramigera.* Author reported that growth was generally better without carbohydrates.

Added nutrients: The author added 0.25% of one of the following:

d-glucose
d-levulose
d-mannose
lactose
starch
raffinose
d-galactose
sucrose
maltose
dextrin
inulin

Reference: Blöch (1918 p. 51).

1355. Kappen's Urea Meat Extract Solution

Constituents:

1. Water	1000.0 cc.
2. Meat extract (1.0%)	10.0 g.
3. Urea (5.0%)	50.0 g.

Preparation:

(1) Dissolve 5.0% urea in a 1.0% meat extract solution.

(2) Distribute in 100.0 cc. lots.

Sterilization: Method not given.

Use: To study decomposition of urea by *Urobacillus Pasteuri, Planosarcina ureae, Bacterium Zopfi* and Cyanamide decomposing bacteria.

Variants: Murray studied ammonification using a 1.0% urea meat infusion solution.

References: Kappen (1909 p. 394), Murray (1916 p. 598).

1356. Viehoever's Urea Meat Extract Solution

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.3 g.
5. NaCl	0.1 g.
6. $FeCl_3$	0.01 g.
7. Urea (2.0%)	20.0 g.
8. Liebig's meat extract (0.5%)	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: To study spore production by Urea splitting organisms, *Bac. probatus.*

Reference: Viehoever (1913 p. 214).

1357. Kappen's Cyanamide Meat Extract Solution

Constituents:

1. Water	1000.0 cc.
2. Meat extract (1.0%)	10.0 g.
3. Cyanamide (0.5%)	5.0 g.

Preparation:

(1) Prepare a 1.0% meat extract solution.

(2) Make slightly alkaline to litmus by the addition of Na_2CO_3.

(3) Add 0.5% Cyanamide to (2).
(4) Distribute in Erlenmeyer flasks.

Sterilization: Method not given.

Use: To study cyanamide decomposition by bacteria.

Reference: Kappen (1909 p. 392).

1358. Homer's Tryptophane Gelatin Solution

Constituents:

1. Water	1000.0 cc.
2. Nutrient salts	
3. Tryptophane (0.15%)	1.5 g.
4. Gelatin (1.0%)	10.0 g.

Preparation:
(1) Add 1.0% gelatin and 0.15% tryoptophane to a solution containing necessary nutrient salts.

Sterilization: Not specified.

Use: Indol production.

Variants: The author added 1.0% glucose.

Reference: Homer (1906 p. 402).

1359. Remy and Rösing's Gelatin Solution

Constituents:

1. Water	1000.0 cc.
2. Gelatin (0.8%)	8.0 g.

Preparation:
(1) Add 0.8% gelatin to 1000.0 cc. water.
(2) Neutralize with soda.
(3) Distribute into small Erlenmeyer flasks.

Sterilization: Sterilize in streaming steam by means of the fractional method.

Use: To study decomposition of organic N materials by soil forms.

Reference: Remy and Rösing (1911 p. 39).

1360. Standfuss and Kallert's Bone Jelly Solution

Constituents:
1. Water.
2. Bone jelly.

Preparation:
(1) Bone jelly is obtained from a factory where fresh bones are rendered under a pressure of 2 to 4 atmospheres in an autoclave. The rendered material is evaporated or condensed to a jelly-like material which is quite stable.
(2) Dissolve some of the jelly in hot water. The amount depends on the consistency of the jelly.
(3) Clarify and filter, obtaining a completely clear golden yellow fluid.
(4) To obtain best results it is well to add some albuminous material. Peptone may be added but a material from the bone jelly gives equally good results. Mix equal parts of water, bone jelly and HCl (strength not specified) and heat in a water bath for 24 hours. Neutralize with soda, clarify and filter.
(5) Add 1.0% of (4) to (3).

Sterilization: Not specified.

Use: Inexpensive culture medium.

Reference: Standfuss and Kallert (1920–21 p. 223).

1361. Berman and Rettger's Gelatin Solution

Constituents:

1. Water	1000.0 cc.
2. Gelatin	2.5 g.
3. NaCl	5.0 g.

Preparation:
(1) Dissolve 2 and 3 in 1. (2.5 g. Liebig's meat extract may be added.)
(2) Tube in 10.0 cc. lots.

Sterilization: Not specified.

Use: To study bacterial nutrition.

Reference: Berman and Rettger (1918 p. 381).

1362. Fleming's Blood and Minced Meat Medium

Constituents:
1. Meat
2. Blood.

Preparation:
(1) Add a little blood to a minced meat medium such as is commonly used to grow anaerobes.

Sterilization: Not specified.

Use: Preservation of *B. influenzae.* Author reported that subcultures from this medium after 6 weeks gave growths as copious as after two days.

Variants: Harvey added a little whole blood to the meat medium as prepared in medium 1239.

References: Fleming (1919 p. 139), Harvey (1921–22 p. 72).

1363. Dean and Nouat's Blood Egg Medium (Cutler)

Constituents:

1. Water	300.0 cc.
2. Egg	1
3. Blood, human	drop

Preparation:

(1) Break up the white and yolk of one egg in a flask containing beads and shake thoroly.

(2) Add 300.0 cc. of water and again shake.

(3) Place the flask in a water bath and bring to boiling point.

(4) Maintain this temperature for 30 minutes, shaking from time to time.

(5) Tube in 5.0 cc. amounts.

(6) Add a few drops of human blood.

Sterilization: Sterilize in the autoclave.

Use: Cultivation of *Entamoeba histolytica* and other intestinal protozoa as amoeba, etc.

References: Cutler (1918 p. 22), Stitt (1923 p. 51).

1364. Bruschettini's Egg Yolk Blood Infusion Solution (Kolle and Wassermann)

Constituents:

1. Veal infusion solution	100.0 cc.
2. Blood, defibrinated, rabbit or dog	10.0 cc.
3. Egg yolk	5.0 cc.

Preparation:

(1) Mix 1, 2 and 3.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

Reference: Kolle and Wassermann (1912 p. 413).

1365. Noguchi's Serum Tissue Medium

Constituents:

1. Distilled water	300.0 cc.
2. Serum	100.0 cc.
3. Tissue, (rabbit)	

Preparation:

(1) Tube a mixture of one part serum (sheep, horse or rabbit) and three parts distilled water in test tubes 20 cm. high and 1.5 cm. wide to a depth of about 16 cm.

(2) Add to each sterile tube a small piece of freshly removed sterile rabbit tissue (testicles, kidney or heart muscle) (liver being unsuitable).

(3) Incubate at 37° for 2 days to test sterility.

Sterilization: Sterilize (1) at 100°C. for 15 minutes on 3 successive days.

Use: Cultivation of *Treponema pallidum* and spirochaetes. The author added a layer of sterile paraffin oil to each tube and incubated under strict anaerobic conditions at 35° to 37°C.

Variants: Pitfield prepared a similar medium as follows:

(1) Obtain dog, sheep or cow blood under aseptic conditions.

(2) Pipette off the serum with a sterile pipette.

(3) Mix 3 to 4 parts of distilled water with 1 part (2).

(4) Tube.

(5) Add a bit of sterile animal tissue to each tube.

References: Noguchi (1911 p. 101), (1911 pp. 1550–1551), Pitfield (1922 p. 119).

1366. Hata's Serum Tissue Medium

Constituents:

1. Physiological salt solution	80.0 cc.
2. Horse serum	40.0 cc.
3. Kidney	

Preparation:

(1) Draw blood from the vein of a normal horse into tall glass cylinders and allow the serum to separate.

(2) Pipette 4.0 cc. of the serum into tubes having a diameter of 1.5 to 1.7 cm.

(3) To each tube add 8.0 cc. of physiological salt solution and mix well. The contents of the tube are about 6.5 to 7.0 cm. high.

(4) Place in a water bath at 58°C. raising the temperature gradually until it reaches 70° or 71°C. in three hours.

(5) Heat for 30 minutes at 71°C. The contents present a translucent milky appearance with a semi-solid surface.

(6) Immerse one or two small pieces of rabbit kidney in each tube. It may be necessary to push the kidney to the bottom by means of a sterile glass rod.

Sterilization: Not specified.

Use: Cultivation of *Spirochaeta recurrens*. Inoculate the medium with infected blood by means of a sterile capillary tube. Author reported that in the kidney medium growth appeared after 24 hours and reached a maximum on the 4th day. In the buff coagulum medium growth was at a maximum at the end of the 5th to 7th days. The number seemed to be less than in the kidney medium. In the buff medium the spirochaetes kept their regular forms of life for a longer period of time and kept their virulence for at least two months.

Variants: In place of the kidney 2 or 3 small pieces of buff coagulum cut in 1 cc. dimensions may be added. Buff coagulum is a part of the blood clot. The clot separates into two layers, the cruor, consisting of red corpuscles and a buff coagulum consisting of white corpuscles and blood platelets. These pieces of buff coagulum must be pushed to the bottom of the medium with a sterile glass rod.

Reference: Hata (1913 p. 109).

1367. Noguchi's Ascitic Fluid Tissue Medium

Constituents:
1. Ascitic fluid.
2. Tissue (rabbit).

Preparation:
(1) Place sterile pieces of tissue, usually rabbit kidney, in test tubes 2 x 20 cm.
(2) After inoculation add quickly about 15.0 cc. of sterile unheated unfiltered ascitic fluid.

Sterilization: Materials are obtained under aseptic conditions.

Use: Cultivation of spirochaetae, *Spirochaeta duttoni*, *Spirochaeta kochi*, *Spirochaeta obermeieri*, *Spirochaeta novyi*, treponemata and others. A few centimeters of citrated blood from the heart of an infected rat or mouse was added to the rabbit tissue before the addition of ascitic fluid. Incubate under anaerobic as well as aerobic conditions. Flexner and Noguchi inoculated the medium with a piece of cerebrum or other part of the brain or spinal cord of an infected animal to cultivate the organism causing epidemic poliomyelitis. Noguchi isolated and cultivated *Spirochaeta gallinarum*, inoculating the medium with a blood emulsion containing the spirochaeta. Loewe and Strauss cultivated the organism causing epidemic encephalitis. Olitsky and Gates isolated and cultivated anaerobic organisms found in influenza, in the medium.

Variants:
(a) Noguchi substituted hydrocele fluid for ascitic.
(b) Stitt gave the following method of preparation, of a medium for the cultivation of treponemata.
  (1) Fit a test tube with a perforated rubber stopper which can be pushed down into the tube.
  (2) Pass a piece of glass tubing thru the stopper to project slightly into the test tube.
  (3) Draw out the other end of the glass tube into a capillary tube and bend at an acute angle.
  (4) Boil the apparatus.
  (5) When cool a piece of sterile tissue is dropped into the tube.
  (6) Draw a strip of gauze thru a glass bead and soak the gauze in the material to be cultured.
  (7) Drop (6) into the bottom of the tube.
  (8) Run in ascitic fluid to the point where it would be reached by the bottom of the rubber stopper.
  (9) Push in the stopper as quickly as possible and when fluid appears in the capillary tube seal off quickly with a small flame.

References: Noguchi (1912 p. 201), Flexner and Noguchi (1913 p. 463), Noguchi (1916 p. 622), Rosenow and Towne (1917 p. 177), Loewe and Strauss (1920 p. 252), Olitsky and Gates (1921 p. 715), Abbott (1921 p. 635), Stitt (1923 p. 53).

1368. Kligler and Robertson's Ascitic Fluid Egg Medium. (Stitt)

Constituents:

1. Egg albumin................	300.0 cc.
2. Ascitic fluid.................	100.0 cc.

Preparation:
(1) Mix three parts egg albumin with one part ascitic fluid (horse or rabbit serum may be used instead).
(2) Tube in tall tubes.

Sterilization: Not specified.

Use: Cultivation of *Borrelia recurrentis*. The medium was covered with a layer of oil not greater than 1.5 cm. high.

Reference: Stitt (1923 p. 54).

1369. Bacto Egg—Meat Medium (Dehydrated)

Constituents:

1. Water	1000.0 cc.
2. Egg albumin	
3. Beef	1.0 lb.
4. $CaCO_3$	5.0 g.

Preparation:

(1) Suspend 150.0 g. of Bacto Egg-Meat Medium (Dehydrated) in 1000.0 cc. of distilled water. (Each liter contains the whites of 6 eggs, 1 pound of lean beef and 5.0 g. $CaCO_3$).

(2) Distribute an even mixture in tubes.

(3) If sterilized at 15 pounds pressure for 20 minutes, pH = 7.2 ±.

Sterilization: Sterilize 20 minutes at 15 pounds, allowing autoclave to lose pressure slowly.

Use: Cultivation of anaerobes.

Reference: Digestive Ferments Co. (1925 p. 11).

1370. Rettger's Egg-Meat Medium

Constituents:

1. Water	1000.0 cc.
2. Beef	1.0 lb.
3. Egg white	
4. $CaCO_3$ (0.5%)	5.0 g.

Preparation:

(1) Thoroly mix 1 pound of lean chopped beef with 500.0 cc. of water.

(2) Neutralize (1) with Na_2CO_3.

(3) Heat in the Arnold sterilizer for 30 minutes, stirring occasionally.

(4) Allow to stand for several hours and remove the fatty scum.

(5) Mix the whites of 6 eggs with 500.0 cc. of water.

(6) Neutralize (5). (Method not given).

(7) Heat in the Arnold for 30 minutes with occasional stirring to coagulate.

(8) Mix (4) and (7) and place into a liter flask.

(9) Add 0.5% of powdered calcium carbonate to (8).

Sterilization: Sterilize at 110 to 112°C. in the autoclave for 30 minutes.

Use: To study putrefaction by *B. coli communis*, *B. lactic aerogenes*, *B. putrificus*, bacillus of malignant oedema, bacillus of symptomatic anthrax. The author replaced the air in the flask with hydrogen gas.

References: Rettger (1907 p. 75), Tanner (1919 p. 58).

1371. Besredka's Egg Meat Infusion Medium (Bezançon)

Constituents:

1. Meat infusion solution	1000.0 cc.
2. Egg.	

Preparation:

(1) Prepare a meat infusion solution (no peptone or NaCl added).

(2) To each 100.0 cc. of sterile (1) contained in Roux flasks add 20.0 cc. of a sterile 10.0% egg white solution and from 5.0 to 20.0 cc. of a sterile 10.0% egg yolk solution under aseptic conditions.

Sterilization: Method not given. The infusion, egg white and egg yolk solutions are sterilized separately.

Use: Cultivation of tubercle bacilli.

Reference: Bezançon (1920 p. 546).

1372. Ficker's Sputum Serum Solution

Constituents:

1. Serum (beef)	200.0 cc.
2. Sputum	100.0 cc.
3. Glycerol (2.0%)	6.0 g.

Preparation:

(1) Mix two parts sterile beef serum with one part sterile tuberculous or bronchitus sputa.

(2) Add 2.0% glycerol to (1).

Sterilization: Sterilize tuberculous or bronchitus sputa for an hour on 3 successive days in streaming steam. Method of sterilization of serum or glycerol not given.

Use: Cultivation of tubercle bacilli.

Reference: Ficker (1900 p. 508).

1373. Kayser's Blood Bile Solution

Constituents:

1. Bile, beef	5.0 cc.
2. Blood	2.5 cc.

Preparation:

(1) Place 5.0 cc. of sterile beef bile into a sterile test tube.

(2) Add about 2.5 cc. of the patient's blood that has been removed from a finger tip or an ear under aseptic conditions.

Sterilization: Method of sterilization of bile not specified.

Use: Enrichment medium for typhoid and paratyphoid used in diagnosis by blood culture method.

Reference: Kayser (1906 p. 186), Klimmer (1923 p. 214).

1374. Noguchi's Blood Serum Solution

Constituents:

1. Serum	100.0 cc.
2. Ringer's Solution	300.0 cc.
3. Plasma, citrated, rabbit	150.0 cc.

Preparation:

(1) Mix 1 part rabbit serum with 3 parts Ringer's solution (see medium 180) and 0.5 part of citrated rabbit plasma.

Sterilization: Not specified.

Use: Cultivation of *Spirochaeta icterohaemorrhagiae*, *Leptospira icterohaemorrhagiae* and other spirochaeteceae. The medium was covered with a layer of paraffin oil following inoculation with suspected blood material.

Variants:

(a) The author used 0.9% NaCl solution instead of Ringer's solution.

(b) The author cultivated *Leptospira icterohaemorrhagiae* on a mixture of 1.5 parts rabbit serum, 4.5 parts Ringer's solution and 1.0 part citrated plasma.

(c) Kaneko cultivated *Spirochaeta icterohaemorrhagiae* and *Spirochaeta hebdomadis* on medium prepared as follows:

(1) Introduce a drop of dog blood into 2.0 to 3.0 cc. of dog serum diluted with 1 to 5 parts Ringer solution.

(2) Heat for 30 minutes at 56 to 58°C.

(3) Cover with liquid sterile paraffin.

References: Noguchi (1917 p. 761), (1918 p. 606), Kaneko (1921–22 p. 354), Abbott (1921 p. 635).

1375. Berman and Rettger's Casein Meat Extract Medium

Constituents:

1. Water	1000.0 cc.
2. Casein	1.25 g.
3. Liebig's meat extract	5.0 g.
4. NaCl	5.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Tube in 10.0 cc. quantities.

Sterilization: Not specified.

Use: To study bacterial nutrition.

Reference: Berman and Rettger (1918 p. 382).

1376. Adams' Coke Milk Medium

Constituents:

1. Milk.
2. Coke.

Preparation:

(1) Add sterile pieces of coke to skimmed milk.

Sterilization: Method not given in the abstract.

Use: Cultivation of butyric acid bacilli. Before use the oxygen was removed by heating and after inoculation with the fecal material the tubes were again heated for 2 to 3 minutes at 70°C. to eliminate the less resistant organisms.

Reference: Adams (1921 p. 59). Taken from (1921 p. 319).

SUBGROUP I-C. SECTION 17

Liquid media or basal solutions not containing digests, but containing extracts of ashes, soil or similar materials.

A_1. Ashed material employed.
  Harrison and Barlow's Basal Wood Ash Medium 1377
  Pasteur's Sucrose Yeast Ash Solution (Smith) 1378
A_2. Soil or soil constituents employed.
B_1. Humic acid or its salts used.
  Krzemieniewska's Glucose Humate Solution (Vogel) 1379
  Söhngen's Salt Humate Solution 1380
B_2. Soil infusions used.
C_1. Containing no additional organic nutrients.
D_1. Inorganic nitrogen added.
  Gutzeit's Ammonium Sulphate Soil Infusion 1381
  Stoklasa's Nitrate Soil Infusion I 1382
  Stoklasa's Nitrate Soil Infusion II 1383
D_2. Inorganic nitrogen not added.
  Löhnis and Pillai's Basal Soil Infusion 1384
  Buchanan's Basal Soil Infusion 1385
  Saltet's Sulphite Mud Solution 1386
  Löhnis' Soil Infusion Medium 1387
  Zikes' Soil Infusion Medium 1388
  Harvey's Soil Infusion Medium 1389
C_2. Containing additional organic nutrients
D_1. Organic nitrogen added.
  Löhnis Urea Soil Infusion Medium 1390

Löhnis and Moll's Asparagin Soil Infusion Medium.................. 1391
Perotti's Dicyandiamid Soil Infusion Medium.................. 1392
D_2. Organic nitrogen not added.
Gutzeit's Mannitol Soil Infusion Medium.......................... 1393
Jensen's Nitrate Soil Infusion Medium.......................... 1394

1377. Harrison and Barlow's Basal Wood Ash Medium

Constituents:

1. Distilled water............. 1000.0 cc.
2. Wood ashes............... 15.0 g.

Preparation:

(1) Add 15.0 g. wood ashes (maple wood, mixed beech and maple, elm and tamarack give good results) to 1000.0 cc. distilled water.

(2) Heat in flowing steam, boil a minute and filter.

Sterilization: Sterilize in flowing steam or in the autoclave at 10 pounds steam pressure for 10 to 20 minutes.

Use: Cultivation of *Pseudomonas radicicola* and other nodule bacteria.

Variants:

(a) Harrison and Barlow added 1.0% or 1.5% maltose to the medium as prepared above.

(b) Harrison and Barlow prepared media as follows:

(1) To 1000 parts cold water add 2.5 to 25 parts by weight (see table) of wood ashes (maple, maple and beech mixed, elm and tamarack gave good results).

(2) Shake well and filter at once or after an hour or heat together to boil, boil a minute and filter. The filtrate should be almost colorless and more alkaline as the amount of ash and as the time before filtration is increased.

(3) To 1000.0 parts of filtrate add 5 to 20 parts of maltose, sucrose or dextrose (see table), maltose being best.

(4) Heat in flowing steam and boil a moment over the flame or heat in the autoclave for 20 minutes or more at 10 pounds steam pressure. This medium will vary faintly alkaline to phenolphthalein to alkaline −0.6 according to the amount of ash used.

(5) If desired acid potassium phosphate may be added 2 to 5 parts per 1000 (see table) at any stage in the preparation but best perhaps after adding the sugar.

(6) Sterilize in flowing steam or in the autoclave at 10 pounds steam pressure for 10 to 20 minutes.

The accompanying table shows the percentages of constituents used and the reaction.

Water..............	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Ash................		1.0	1.0	1.0	1.0	1.5	1.0	2.0	1.0	1.0	2.0	1.0	1.0	1.0	1.0	2.0
Maltose............					2.0		1.0	1.0	2.0	1.0	2.0			2.0	0.5	1.0
Sucrose............			2.0	2.0								2.0	1.0		2.0	
KH_2PO_4...........				0.5	0.2	0.5	2.0	2.0						0.5	0.5	1.0
To litmus..........		−11°	−10°	−11°	+4°	Alk.	+28°	+28°	−5°	Neut.	Neut.	−5°	−5°	+	+	+2°
To phthalein.......		−8°	−6°	+31°	+19°		+93°	+63°	−2°	Neut.	Neut.	−2°	−5°	+	+	+18°

(c) Percival prepared the medium as follows:

(1) Add 8.0 g. of well burnt wood ashes to 500.0 cc. distilled water and boil for one minute.

(2) Filter thru two sheets of paper.

(3) Add 4.0 g. of maltose to 400.0 cc. of the filtrate, and boil.

(4) Tube and sterilize in the usual way, method not given.

(d) Giltner gave the following method of preparation of the medium:

(1) Stir 5.0 g. of wood ashes (elm, beech, maple) into 1000.0 cc. of distilled water for 2 or 3 minutes only.

(2) Filter.

(3) Add 1.0% commercial sucrose.

(4) Boil for 5 minutes over a free flame.

(5) Strain while hot thru several thicknesses of clean cheese cloth. This may be filtered if desired.

(6) Sterilize by the Tyndall method in the steamer.

References: Harrison and Barlow (1907 pp. 267, 269), Percival (1920 p. 204), Giltner (1921 p. 377).

1378. Pasteur's Sucrose Yeast Ash Solution (Smith)

Constituents:

1. Distilled water	1000.0 cc.
2. Ammonium tartrato	10.0 g.
3. Yeast ash	10.0 g.
4. Sucrose	100.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1 in the cold.

Sterilization: Not specified.

Use: Cultivation of yeast.

Variants:

(a) Roux and Rochaix, and Bezançon, used 0.75 g. yeast ash and omitted the ammonium tartrate.

(b) Kolle and Wassermann used the ashes from 10.0 g. yeast.

(c) Tanner, Besson, Bezançon, Dopter and Sacquépée, used 1.0 g. ammonium tartrate and used 0.75 g. yeast ashes.

References: Smith (1905 p. 197), Roux and Rochaix (1911 p. 103), Kolle and Wassermann (1912 p. 393), Tanner (1919 p. 66), Besson (1920 p. 36), Bezançon (1920 p. 115) Dopter and Sacquépée (1921 p. 121), Harvey (1921–22 p. 120).

1379. Krzemieniewska's Glucose Humate Solution (Vogel)

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4 \cdot 7H_2O$	0.125 g.
3. $CaHPO_4 \cdot 2H_2O$,	0.25 g.
4. Sodium humate	10.0 cc.
5. Glucose	9.0 g.

Preparation:

(1) Prepare a sodium humate solution by shaking 1000.0 g. of humus soil in a dilute solution of NaOH.

(2) Filter thru glass wool.

(3) Acidify the filtrate with HCl.

(4) Wash the precipitate until the wash water shows a very weak acid reaction.

(5) Dissolve the filtrate in weak NaOH solution.

(6) Dissolve 2, 3 and 5 in 1.

(7) Add 10.0 cc. of (5) to (6).

Sterilization: Not specified.

Use: To study nitrogen assimilation by azotobacter. Vogel reported little difference in amount of nitrogen assimilated whether K_2SO_4 be present or not. Possibly there was slightly more nitrogen assimilated per mg. of glucose utilized if the potassium salt was not added.

Variants: The author added 0.25 g. K_2SO_4 to the medium.

Reference: Vogel (1911–12 p. 419).

1380. Söhngen's Salt Humate Solution

Constituents:

1. Distilled water	1000.0 cc.
2. $MgSO_4$	0.5 g.
3. Bipotassium phosphate	0.5 g.
4. NH_4Cl	0.5 g.
5. Sodium humate	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of paraffin and petroleum oxidizers, *Mycobacterium*, *B. fluorescens liquefaciens* and *Micrococcus paraffinae*. Author reported that sodium humate was utilized by these organisms.

Reference: Söhngen (1913 p. 603).

1381. Gutzeit's Ammonium Sulphate Soil Infusion

Constituents:

1. Water	1000.0 cc.
2. Soil	
3. $(NH_4)_2SO_4$ (1.0%)	10.0 g.
4. $CaCO_3$ (0.5%)	5.0 g.

Preparation:

(1) Prepare soil extract.

(2) Add 1.0% $(NH_4)_2SO_4$ and 0.5% $CaCO_3$ to (1).

Sterilization: Not specified.

Use: To study denitrification.

Variants: Löhnis prepared a similar medium as follows:

(1) Heat 1000.0 g. of good rich garden soil with a liter of tap water for 30 minutes in the autoclave under pressure of 1 atmosphere or boil with 2 liters of water over a free flame.

(2) Pour off the turbid liquid.

(3) Mix talc with the liquid.

(4) Filter thru a double filter paper.

(5) Make up the volume to 800.0 cc. if necessary.

(6) Dissolve 1.0% $(NH_4)_2SO_4$ and 0.5% K_2HPO_4 in (5).
(7) Add chalk after inoculation with soil.
(8) Sterilization not specified.

Reference: Gutzeit (1906 p. 370), Löhnis (1913 p. 110).

1382. Stoklasa's Nitrate Soil Infusion I

Constituents:

1. Distilled water	
2. Soil	9000.0 g.
3. $NaNO_3$	1.5 g.
4. K_2HPO_4	1.0 g.
5. $MgSO_4$	1.0 g.

Preparation:

(1) Extract 9000.0 g. of soil with distilled water at 60°C. (Amount of water or time not specified.)
(2) Concentrate the extract (19 liters) to 1 liter. This liter contains 2.2 g. of carbon in the form of soluble organic compounds.
(3) Add 1.5 g. $NaNO_3$, 1.0 g. K_2HPO_4 and 1.0 g. $MgSO_4$ to 600.0 cc. of (2).

Sterilization: Sterilize four times in the steamer.

Use: To study denitrification by *Bact. Hartlebi*. Author reported no denitrification.

Reference: Stoklasa (1907 p. 32).

1383. Stoklasa's Nitrate Soil Infusion II

Constituents:

1. Distilled water	
2. Soil	2500.0 g.
3. $NaNO_3$	0.5 g.
4. $CaCO_3$	2.5 g.

Preparation:

(1) Extract 2500.0 g. soil with distilled water three times (15 liters of water in all).
(2) Evaporate to 160.0 cc. This extract contains 2.55 g. organic carbon per liter (determined by oxidation with chromic acid).
(3) Add 0.5 g. $NaNO_3$, containing 0.08 g. N and 2.5 g. $CaCO_3$ to 150.0 cc. of (2).

Sterilization: Sterilize four times in the steamer.

Use: To study denitrification by *Bact. Hartlebi*. Author reported no denitrification.

Reference: Stoklasa (1907 p. 33).

1384. Löhnis and Pillai's Basal Soil Infusion

Constituents:

1. Water	1000.0 cc.
2. Soil	1000.0 g.
3. K_2HPO_4 (0.5%)	5.0 g.
4. $CaCO_3$ (1.0 to 2.0%)	10.0 to 20.0 g.

Preparation:

(1) Add 1000.0 g. soil to 1 liter of water.
(2) Place in a covered pot and autoclave for 30 minutes at 1 atmosphere pressure.
(3) Filter. One obtains about 600.0 cc.
(4) To (3) add 0.5% K_2HPO_4 and 1.0% of the added nutrients.
(5) Add 1.0 to 2.0% $CaCO_3$.

Sterilization: Not specified.

Use: To study N assimilation. Author reported that more nitrogen was assimilated with mannitol than glucose, $CaCO_3$ favored the nitrogen assimilation with sugar but not with the acids.

Variants: The author omitted the $CaCO_3$.

Added nutrients: The author added 1.0% of one of the following:

mannitol
glucose,
tartaric acid (neutralized with soda)

Reference: Löhnis and Pillai (1907 p. 88).

1385. Buchanan's Basal Soil Infusion

Constituents:

1. Water	1000.0 cc.
2. Soil	100.0 g.

Preparation:

(1) Boil 100.0 g. of rich garden soil with 1 liter of water.
(2) Filter until clear.
(3) Dissolve 2.0% of one of the added nutrients in (2).

Sterilization: Sterilize for 20 minutes on each of 3 successive days in streaming steam.

Use: To study gum production.

Added nutrients and variants: The author added 2.0% of one of the following:

glucose
levulose
galactose
maltose
sucrose
lactose
mannitol

Reference: Buchanan (1909 p. 388).

1386. Saltet's Sulphite Mud Solution

Constituents:

1. Water (ditch).

2. Mud.
3. Na_2SO_3.

Preparation:

(1) Mix ditch water and mud.
(2) Distribute in test tubes.
(3) To each tube of sterile (2) add two drops of a 10.0% Na_2SO_3 solution.

Sterilization: Sterilize (2) at 110°C.

Use: To study sulphite reduction by *B. desulfuricans*.

Variants: The author added two drops of a 10.0% $Na_2S_2O_3$ solution to each tube instead of Na_2SO_3.

Reference: Saltet (1900 p. 697).

1387. Löhnis' Soil Infusion Medium

Constituents:

1. Water (tap)	1000.0 cc.
2. Soil	1000.0 g.

Preparation:

(1) Heat 1000.0 g. of good rich garden soil with a liter of tap water for 30 minutes in the autoclave under pressure of 1 atmosphere or boil with 2 liters of water over a free flame.
(2) Pour off the turbid liquid.
(3) Mix talc with the liquid.
(4) Filter thru a double filter paper.
(5) Make up the volume to 800.0 cc. if necessary.

Sterilization: Method not given.

Use: Cultivation of soil microörganisms.

Variants:

(a) Sherman boiled one part soil with 3 parts water, filtered until clear and then added a small excess of $CaCO_3$. He reported that this medium was especially adapted for the cultivation of flagellates, ciliates and amoebae.
(b) Giltner used 2 liters of water to 1000.0 g. soil.

References: Löhnis (1913 p. 101), Sherman (1916 p. 42), Giltner (1921 p. 370), Harvey (1921–22 p. 105), Cunningham (1924 p. 136).

1388. Zikes' Soil Infusion Medium

Constituents:

1. Water	1000.0 cc.
2. Soil	100.0 g.
3. KH_2PO_4 (0.3%)	3.0 g.
4. $MgSO_4$ (0.2%)	2.0 g.

Preparation:

(1) Extract 100.0 g. of soil with 1000.0 cc. water by boiling.
(2) Filter.
(3) Add 0.3% KH_2PO_4 and 0.2% $MgSO_4$.

Sterilization: Not specified.

Use: Cultivation of *Apiculatus yeast, Torula alba, Torula Molischiana, Mycoderma cerevisiae Blastoderma salmonicolor.*

Reference: Zikes (1911 p. 147).

1389. Harvey's Soil Infusion Medium

Constituents:

1. Water (tap)	1000.0 cc.
2. Soil	100.0 g.
3. K_2HPO_4	0.5 g.

Preparation:

(1) Boil 100.0 g. of rich garden soil in 200.0 cc. of water.
(2) Pour off the turbid liquid and add a little talc.
(3) Filter until clear thru thick filter paper.
(4) Add 900.0 cc. of tap water and 0.5 g. K_2HPO_4 to 100.0 cc. of (3).

Sterilization: Not specified.

Use: Cultivation of flagellates and ciliates from the soil.

Reference: Harvey (1921–22 p. 108).

1390. Löhnis Urea Soil Infusion Medium

Constituents:

1. Water	1000.0 cc.
2. Soil	1000.0 g.
3. K_2HPO_4 (0.05%)	0.5 g.
4. Urea (5.0%)	40.0 g.

Preparation:

(1) Prepare soil infusion as indicated in medium 1387.
(2) Dissolve 0.05% K_2HPO_4 and 5.0% urea in (1).

Sterilization: Not specified.

Use: Cultivation of organisms capable of utilizing urea.

Reference: Löhnis (1913 p. 108), Giltner (1921 p. 374).

1391. Löhnis and Moll's Asparagin Soil Infusion Medium

Constituents:

1. Water	1000.0 cc.
2. Soil	500.0 g.
3. K_2HPO_4 (0.5%)	5.0 g.
4. Glucose (0.1%)	1.0 g
5. Asparagin (0.1%)	1.0 g

Preparation:

(1) Allow 50.0% soil to stand in water for 20 hours and then filter.

(2) Dissolve 2, 3 and 4 in (1).

Sterilization: Medium may or may not be sterilized.

Use: Cultivation of organisms capable of utilizing calcium cyanamide.

Variants:

(a) The authors added 0.014 g. of calcium cyanamide.

(b) Löhnis prepared the medium by dissolving 0.2% calcium cyanamide, 0.01% glucose, 0.05% K_2HPO_4 and 0.01% asparagin in soil infusion prepared as in medium 1387.

References: Löhnis and Moll (1907 p. 265), Löhnis (1913 p. 108).

1392. Perotti's Dicyandiamid Soil Infusion Medium

Constituents:

1. Water	2000.0 cc.
2. Garden soil	1000.0 g.
3. Dicyandiamid	2.0 g.
4. K_2HPO_4	0.5 g.
5. Glucose	0.1 g.

Preparation:

(1) Boil 1000.0 g. garden soil with 200.0 cc. of water for 2 hours.

(2) Concentrate the liquid a little and filter.

(3) Dilute until the filtrate contains 0.4% mineral substances.

(4) Add 3, 4 and 5 to (3).

Sterilization: Not specified.

Use: Cultivation of organisms utilizing dicyandiamid from the soil.

Reference: Perotti (1908 p. 207).

1393. Gutzeit's Mannitol Soil Infusion Medium

Constituents:

1. Water	1000.0 cc.
2. Soil	
3. Mannitol (1.0%)	10.0 g.

Preparation:

(1) Dissolve 1.0% mannitol in a soil infusion.

Sterilization: Not specified.

Use: Gutzeit used the medium to study denitrification. Löhnis and Cunningham used similar media for the enrichment of nodule bacteria and azotobacter.

Variants: Löhnis added 1.0% mannitol and 0.05% K_2HPO_4 to soil infusion (see medium 1387) and sterilized in the autoclave.

References: Gutzeit (1906 p. 370), Löhnis (1913 p. 112), Cunningham (1924 p. 155).

1394. Jensen's Nitrate Soil Infusion Medium

Constituents:

1. Water	1000.0 cc.
2. Soil	500.0 g.
3. $NaNO_3$	20.0 g.
4. Glycerol	

Preparation:

(1) Add 1000.0 cc. of 2.0% solution of $NaNO_3$ to 500.0 g. of soil that has been passed thru a 0.5 mm. sieve.

(2) Add 0.0, 5.0, 20.0 or 50.0 g. of glycerol to (1).

Sterilization: Not specified.

Use: To study denitrification by soil forms. Author reported that the addition of glycerol aided denitrification. No denitrification occurred without glycerol at the end of 14 days.

Reference: Jensen (1897 p. 690).

GROUP II. LIQUEFIABLE AGAR MEDIA

SUBGROUPS OF LIQUEFIABLE AGAR MEDIA

A_1. Water and agar only.
Subgroup II A. (Med. 1395–1401)
A_2. Water and agar with other constituents.
B_1. All other constituents inorganic.
Subgroup II B. (Med. 1402–1430)
B_2. Containing one or more organic constituent (other than agar).
Subgroup II C. (Med. 1431–2198)

SUBGROUP II-A

Media Consisting of Water and Agar Only

Basal or complete media containing agar and water only. Here may be included methods used for purifying agar for use in media. It is rarely used without the addition of other constituents.

A_1. Agar and water only. (Agar not purified.)
Tischutkin's Agar Solution......... 1395
Pringsheim's Basal Agar........... 1396
A_2. Agar purified by washing with water.
Beijerinck's Basal Agar Solution (Smith)......................... 1397
Ayers, Mudge and Rupp's Washed Agar............................ 1398
A_3. Agar purified by washing with alcohol, acids, etc.
Dominikiewicz's Purified Agar...... 1399
Cunningham's Purified Agar....... 1400
Harvey's Purified Agar............. 1401

1395. Tischutkin's Agar Solution

Constituents:
1. Water...................... 1000.0 cc.
2. Agar (1.0%)............... 10.0 g.

Preparation:
(1) Dissolve 1.0% agar (best quality) in water by steaming in the autoclave under 2 atmospheres pressure for 10 minutes.
(2) Filter thru paper.
(3) Tube.

Sterilization: Sterilize in the autoclave under one atmosphere pressure for 15 minutes.

Use: Cultivation of amoebae, algae, parasitic flagellata and ciliata. Frost used a similar medium to study the antagonistic action of bacteria.

Variants:
(a) Frost studied the antagonistic action between saprophytic organisms and *B. typhosus* in medium prepared by the following method:
(1) Dissolve 2.0% agar in water.
(2) Sterilize.
(3) Pour in deep Petri dish to cool.
(4) When solid, cut in little squares with sterile knife.
(5) Stab with needle seeded with organism to be studied.
(6) Seal top of block with red hot nail head.
(7) Place by means of sterile forceps into broth culture of other organism.
Frost reported that if there was antagonistic action, no growth occurred in the block.
(b) Walker cultivated parasitic flagellata and ciliata in a 2.0% agar solution adjusted to an alkalinity of 1.0% to phenolphthalein.

References: Tischutkin (1897 p. 185), Frost (1904 p. 604), Walker (1908 p. 490).

1396. Pringsheim's Basal Agar

Constituents:
1. Water...................... 1000.0 cc.
2. Agar....................... 2.5 g.

Preparation: (1) Dissolve 2 and one of the added nutrients in 1.

Sterilization: Not specified.

Use: To study nitrogen assimilation by *Clostridium americanum* and *Azotobacter chroococcum* with *Bacillus gelaticus.* Author reported that *Bacillus gelaticus* with *Clostridium americanum* assimilated more nitrogen than did *Bacillus gelaticus* with *Azotobacter chroococcum.*

Added nutrients and variants: The author prepared one of the following media, using water and varying amounts of agar as a base:

(a) 2.5 g. agar and 0.5 g. glucose per 1000.0 cc. water.
(b) 2.0 g. agar and 0.05 g. $(NH_4)_3PO_4$ per 1000.0 cc. water.
(c) 5.0 g. agar + 1.0 g. mannitol per 1000.0 cc. water.
(d) 5.0 g. agar + 0.4 g. mannitol per 1000.0 cc. water.

Author used *Bacillus gelaticus* with *Clostridium americanum* with (a) and (b), and *Bacillus gelaticus* with *Azotobacter chroococcum* in solution (c) and (d).

Reference: Pringsheim (1910 p. 230).

1397. Beijerinck's Basal Agar Solution (Smith)

Constituents:
1. Distilled water.
2. Agar.

Preparation:
(1) Add ordinary agar to distilled water and heat until solution is complete.
(2) Pour into Erlenmeyer flasks and allow to solidify.
(3) When cold, fill the flasks with distilled water and set away.
(4) Change the water several times, during a lapse of one or two weeks.
(5) Add any desired inorganic nutrients and some pure precipitated $CaCO_3$.

Sterilization: Method not given.

Use: Cultivation of nitrite bacteria.

Added nutrients: Smith specified the addition of any solution of nutrient salts containing ammonium salts and states that Beijerinck recommended NH_4, $NaHPO_4 \cdot H_2O$ as the best ammonium salt to be used.

Reference: Smith (1905 p. 199).

1398. Ayers, Mudge and Rupp's Washed Agar

Constituents:
1. Distilled water............. 2000.0 cc.
2. Agar....................... 30.0 g.

Preparation:
(1) Place 30.0 g. of shredded agar in a flask with 2000.0 cc. distilled water.
(2) Allow to stand for 24 hours at room temperature.
(3) Pour off as much water as possible by placing a piece of cheese cloth over the top of the flask.
(4) Add fresh distilled water to make up for water poured off.
(5) Allow to soak for another 24 hours.
(6) Filter thru a cotton flannel cloth and wash once with a liter of distilled water.
(7) Allow the agar to drain as much as possible. Press out the remaining water by squeezing the filter cloth with the hands.
(8) The washed agar may be dissolved in water for the preparation of media or dried in air and used as ordinary agar. In this case use 1.0% of the dry material to obtain a medium having the same jelly strength as a 1.5% shred agar medium.

Sterilization: Not specified.

Use: Purified agar. Giltner used a similar medium in the preparation of a solid synthetic medium and for testing food requirements and selective power of bacteria.

Variants: Giltner used the agar, prepared in the following manner:
(1) Place 15 parts agar in 1000.0 parts distilled water.
(2) Cover the mouth of the bottle with parchment paper, or several layers of clean cheese cloth.
(3) Allow to ferment spontaneously.
(4) Change the water in the bottle occasionally, replacing the amount removed with the same amount of clean distilled water.
(5) When active fermentation has ceased as noted by the evolution of gas place the agar in an agate ware pail.
(6) Weigh.
(7) Boil over a free flame to dissolve the agar.
(8) Make up any loss in weight by the addition of distilled water.
(9) Distribute as desired.
(10) Autoclave.

References: Ayers, Mudge and Rupp (1920 p. 591), Giltner (1921 p. 365).

1399. Dominikiewicz's Purified Agar

Constituents:
1. Water.............. 200.0 to 400.0 cc.
2. Agar............... 30.0 to 40.0 g.

Preparation:
(1) Dissolve 30.0 to 40.0 g. of agar in 200 to 400.0 cc. of water.
(2) Add slowly, 700–800 cc. of alcohol that has been acidified with CH_3COOH.
(3) Shake the mixture thoroly and allow the precipitated agar to settle.
(4) Pour off the alcohol and place the agar precipitate on a Bücher funnel.
(5) Wash the precipitate until the wash water is neutral.
(6) The agar may be washed on a piece of strain cloth instead of a Bücher funnel.
(7) After the alcohol has been removed from the agar, the agar is dried at 100°C.
(8) The dried agar is a snow white powder.

Use: A purified form of agar. When this powdered agar is used in the preparation of various media, it requires no filtration.

Reference: Dominikiewicz (1908 p. 668).

1400. Cunningham's Purified Agar

Constituents:
1. Water.
2. China grass.

Preparation:
(1) Weigh a quantity of china grass and place in a vessel containing sufficient dilute acid (0.01% H_2SO_4 gives the best results) to completely cover the fibre.
(2) Soak in the acid for 10 minutes.
(3) Remove and wash in running water until all traces of acid have been removed. Both the fibre and washings are to be alkaline to litmus.
(4) Express the superfluous water by placing the fibre in a stout cloth and subjecting it to a pressure by twisting the ends in opposite directions.
(5) Place a layer of cotton wool on a layer of butter muslin. Moisten and remove the superfluous water by careful wringing.
(6) Fit (5) to the filter funnel with the cotton up.
(7) Pile the agar (4) on the funnels, and then the funnels are placed in a position over a series of tiltering tins.
(8) Place in the autoclave, and autoclave at 120°C. for 1 hour.
(9) Cool by placing the tins in cold water. The agar sets into a very firm jelly which is cut into slices which are broken up by a meat mincing machine with a very fine bore.
(10) Place the small vermicular threads obtained from (9) in trays, and desiccate in a drying oven at a moderate heat. When the threads are dry they have a light brown color.

Use: A purified agar. A 1.0% solution of the agar gives a clear firm medium.

Variants: The threads may be milled into a fine powder.

Reference: Cunningham (1918–19 p. 561).

1401. Harvey's Purified Agar.

Constituents:
1. Water........................ 200.0 g.
2. Agar........................ 100.0 g.

Preparation:
(1) Mix 1.0 part fibre agar, 0.05 part glacial acetic acid and 2.0 parts water.
(2) Allow the agar to soak in the acidified water 15 minutes.
(3) Remove the fibre agar and wash thoroly with water until quite free from any trace of acid reaction to litmus paper.
(4) Squeeze in a cloth to get rid of excess of water.

Use: Used in the preparation of media, to give a 3% solution of agar, calculating from the weight of the agar in the original dry condition.

Variants: Harvey treated the agar obtained above as follows:
(1) Place the fibre on a filter funnel furnished with moistened cotton wool for filtration.

Note: The filtering wool is supported on butter muslin and the free edges of the latter are turned over the rim of the funnel and tied there. This prevents the wool from sinking down into the narrow part of the funnel and blocking the filtration.

(2) Place the filter funnel with agar in an autoclave over a collecting receptacle.
(3) Keep one hour at 120°C.

(4) Cool rapidly the receptacle, now containing the cleaned filtered agar.
(5) Remove the agar when set.
(6) Mince finely by passing the agar thru a mincing machine furnished with a disc having small holes.
(7) Spread the vermicular threads obtained in shallow layers on trays.
(8) Desiccate in a drying oven at moderate temperature.
(9) Reduce the desiccated material to powder.
(10) Preserve in the dry state.
(11) Use for the preparation of media in a strength of one per cent.

Reference: Harvey (1921–22 p. 66, 67).

SUBGROUP II-B

Agar Media with All Other Constituents Inorganic

Basal or complete media containing agar; all other constituents inorganic.

Key to the Sections of Subgroup II B

A_1. Nitrogen present as free or elementary nitrogen....Section 1 (Med. 1402–1410)
A_2. Nitrogen supplied as ammonium salts Section 2 (Med. 1411–1424)
A_3. Nitrogen supplied as nitrites or nitrates.......Section 3 (Med. 1425–1430)

SUBGROUP II-B. SECTION 1

Basal or complete media containing agar with inorganic salts. Nitrogen present as free or atmospheric nitrogen only.

A_1. Containing salts of monovalent cations only.
  Davis' Sodium Chloride Agar...... 1402
  Beijerinck's Thiosulphate Agar.... 1403
A_2. Containing salts of monovalent and other cations.
B_1. Calcium salts added.
  Gordon's Basal Salt Agar.......... 1404
  Carrel and Burrows' Locke's Solution Agar........................ 1405
  Omeliansky's Basal Carbonate Agar. 1406
  Ashby's Salt Agar (Murray)....... 1407
  Winogradski's Salt Agar (Murray).. 1408
B_2. Calcium salts not added.
  Liot's Basal Salt Agar.............. 1409
  Dox's Salt Agar (Tanner).......... 1410

1402. Davis' Sodium Chloride Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. NaCl	5.0 g.

Preparation: (1) Dissolve 2 and 3 in 1. (Pure or impure sugars may be added.)

Sterilization: Not specified.

Use: To produce chlamydospores by *Sporothrix schenckii*. Smith and Smillie used a similar medium to obtain coccidia spores from sparrow feces. Teague and Deibert studied the constituents necessary for the growth of Unna-Ducrey's bacillus and reported no growth on the medium used.

Variants: Smith and Smillie, and Teague and Deibert dissolved 2.0% agar in a 0.5% aqueous NaCl solution.

References: Davis (1914 p. 485), Smith and Smillie (1917 p. 415), Teague and Deibert (1922 p. 70).

1403. Beijerinck's Thiosulphate Agar

Constituents:

1. Water	1000.0 cc.
2. $Na_2S_2O_3$, $5H_2O$	5.0 g.
3. K_2HPO_4	0.1 g.
4. $NaHCO_3$	0.2 g.
5. Agar	20.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Isolation of *Thiobacillus denitrificans* and *T. thioparus*.

Reference: Beijerinck (1903–04 p. 599).

1404. Gordon's Basal Salt Agar

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4 (0.1%)	1.0 g.
3. $MgSO_4$ (0.2%)	2.0 g.
4. $CaCl_2$ (0.01%)	0.1 g.
5. NaCl (0.5%)	5.0 g.
6. Agar (3.0%)	30.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 3.0% of a thoroughly washed and then dried agar in 1.
(2) Dissolve one of the added nutrients in (1).

Sterilization: Not specified in the abstract.

Use: To determine nitrogen requirement of some of the commoner pathogenic bacteria.

Added nutrients: Gordon added various nitrogen compounds. The compounds used or concentration not specified.

Reference: Gordon (1917 p. 371) taken from (1917 p. 299).

1405. Carrel and Burrows' Locke's Solution Agar

Constituents:

1. Locke's solution	1000.0 cc.
2. Agar (2.0%)	20.0 g.

Preparation: (1) Dissolve 2.0% agar in Locke's solution (see medium 6 for Locke's solution).

Sterilization: Not specified.

Use: Cultivation of tissue in vitrio. Author reported that the medium did not support growth nearly as well as did plasma.

Reference: Carrel and Burrows (1911 p. 245).

1406. Omeliansky's Basal Carbonate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. Na_2CO_3	1.0 g.
4. K_2HPO_4	trace

Preparation: (1) Dissolve 2, 3, 4 and one of the added nutrients in 1.

Sterilization: Not specified.

Use: To study oxidation of sulphites and phosphites. The author reported that neither the phosphite nor sulphite were oxidized.

Added nutrients: The author added 0.3 g. of one of the following:

KNO_2, KNO_3, Peptone.

Variants: The author added 0.0 or 2.0 g. of Na_2SO_3 or Na_2HPO_3.

Reference: Omeliansky (1902 p. 64).

1407. Ashby's Salt Agar (Murray)

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	0.2 g.
3. $MgSO_4$	0.2 g.
4. NaCl	0.2 g.
5. $CaSO_4$	0.2 g.
6. $CaCO_3$	5.0 g.
7. Agar	15.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: To study nitrogen fixation and to determine number of organisms in soil. Used both anaerobically and aerobically.

Reference: Murray (1916 p. 608).

1408. Winogradski's Salt Agar (Murray)

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $MgSO_4$	3.0 g.
4. NaCl	0.01 g.
5. $MnSO_4$	0.01 g.
6. $CaCO_3$	10.0 g.
7. $FeCl_3$ (10% soln.)	2 drops
8. Agar	15.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: To study nitrogen fixation and to determine the number of bacteria in the soil. Used anaerobically and aerobically.

Reference: Murray (1916 p. 608).

1409. Liot's Basal Salt Agar

Constituents:

1. Double distilled water	1000.0 cc.
2. Sodium phosphate	5.0 g.
3. $MgSO_4$	2.5 g.
4. Agar	25.0 g.

Preparation:

(1) Macerate 25.0 g. of commercial agar in 750.0 cc. of double distilled water for 48 hours.

(2) Dissolve in the autoclave at about 100°C.

(3) Filter while hot on a Chardin filter paper.

(4) Dissolve 5.0 g. sodium phosphate in 125.0 cc. of double distilled water.

(5) Dissolve 2.5 g. $MgSO_4$ in 125.0 cc. double distilled water.

(6) Add sterile (4) to (3). Mix well.

(7) Add sterile (5) to (6). Mix well.

(8) Tube in 10.0 cc. quantities.

(9) When ready for use add 0.5 cc. of a 1.0% sterile solution of one of the added nutrients to each sterile tube. (The sodium phosphate and $MgSO_4$ may be omitted, leaving a non-mineral agar.)

Sterilization: Sterilize (4) and (5) at 115° for 15 minutes. Sterilize (8) in the usual manner. (Method not given.) Method of sterilization of the added nutrients not given.

Use: To study pigment production by pyocyanius bacilli.

Added nutrients: The author added 0.5 cc. of a 1.0% solution of one of the following to each 10.0 cc. of the medium:

- Ammonia salts of organic acids
  - Amides
  - Monobasic acids
  - Polybasic acids
  - Amines
- Ammonia salts of inorganic acids
  - Polyatomic alcohols
  - Monosaccharides
  - Polysaccharides
  - Monoses

Reference: Liot (1923 p. 238).

1410. Dox's Salt Agar (Tanner)

Constituents:

1. Distilled water	3000.0	cc.
2. $MgSO_4$	1.5	g.
3. K_2HPO_4	3.0	g.
4. KCl	1.5	g.
5. $FeSO_4$	0.03	g.
6. Agar to solidify		

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Solidify by the addition of agar.

Sterilization: Not specified.

Use: Cultivation of fungi.

Reference: Tanner (1919 p. 65).

SUBGROUP II-B. SECTION 2

Basal or complete media containing agar with inorganic salts. Nitrogen supplied as ammonium salts.

A_1. Ammonia present as ammonium chloride.

Beijerinck's Thiosulphate Ammonium Chloride Agar.............. 1411
Waksman's Thiosulphate Ammonium Chloride Agar.............. 1412
Beijerinck and van Delden's Ammonium Chloride Agar.............. 1413

A_2. Ammonia present as ammonium nitrate.

Munter's Basal Ammonium Agar... 1414
Beijerinck's Ammonium Nitrate Agar 1415

A_3. Ammonia present as ammonium sulphate.

Pesch's Basal Ammonium Sulphate Agar.............................. 1416
Fremlin's Ammonium Sulphate Agar. 1417
Nencki, Sieber and Wynikiwicz's Ammonium Sulphate Agar....... 1418
Gowda's Ammonium Sulphate Agar. 1419
Heinemann's Ammonium Sulphate Agar.............................. 1420
Committee S. A. B. Basal Ammonium Phosphate Agar............ 1421

A_4. Ammonia present as salts of phosphoric acid.

Beijerinck's Sodium Ammonium Phosphate Agar.................. 1422
Stutzer's Ammonium Magnesium Phosphate Agar.................. 1423
Carper's Basal Ammonium Phosphate Agar...................... 1424

1411. Beijerinck's Thiosulphate Ammonium Chloride Agar

Solidify Beijerinck's Thiosulphate Solution, see medium 76 by the addition of 2.0% agar.

1412. Waksman's Thiosulphate Ammonium Chloride Agar

Constituents:

1. Distilled water	1000.0	cc.
2. $Na_2S_2O_3 \cdot 5H_2O$	5.0	g.
3. KH_2PO_4	3.0	g.
4. NH_4Cl	0.1	g.
5. $MgCl_2$	0.1	g.
6. $CaCl_2$	0.25	g.
7. Agar	20.0	g.

Preparation: Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Sterilize at 15 pounds pressure for 15 minutes.

Use: Cultivation and isolation of *Th. thioöxidans.* Author reported that this organism can grow at pH = 1.0. Maximum pH was between 3.0 and 4.0.

Reference: Waksman (1922 p. 607).

1413. Beijerinck and van Delden's Ammonium Chloride Agar

Constituents:

1. Distilled water	1000.0	cc.
2. Agar	15.0	g.
3. K_2HPO_4	0.1	g.
4. NH_4Cl	0.1	g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Isolation of *Bacillus oligocarbophilus.* Author reported that colonies were dry, snowy white or rose colored. If the agar plate be inoculated with straw or an impure culture of the organism, a pure culture may be obtained.

Reference: Beijerinck and van Delden (1903 p. 37).

1414. Münter's Basal Ammonium Nitrate Agar

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4$	0.5 g.
3. NaCl	0.5 g.
4. K_2HPO_4	1.5 g.
5. NH_4NO_3	1.5 g.
6. $CaCl_2$	0.2 g.
7. $FeCl_3$	trace
8. Agar 1.25%	12.5 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8 and one of the added nutrients in 1.

(2) Make slightly alkaline by the addition of Na_2CO_3.

Sterilization: Not specified.

Use: Cultivation of actinomycetes. The author reported that oxalic acid, tartaric acid and hippuric acid were not suitable carbon sources for the actinomycetes studied. The remaining acids were generally fair carbon sources for most of the actinomycetes studied.

Added nutrients: The author added one of the following materials:

acetic acid
lactic acid
oxalic acid
succinic acid
malic acid
aspartic acid
citric acid
hippuric acid
uric acid
humus acid

Reference: Münter (1913 p. 371).

1415. Beijerinck's Ammonium Nitrate Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	20.0 g.
3. NH_4NO_3	0.5 g.
4. Potassium phosphate	0.2 g.
5. $MgSO_4$	0.2 g.
6. $CaCl_2$	0.1 g.

Preparation:

(1) Wash agar with distilled water for some time to separate the soluble material.

(2) Dissolve 3, 4, 5 and 6 in 1.

(3) Dissolve 2.0% (dry) (1) in (2).

(4) Pour into plates.

Sterilization: Not specified.

Use: Isolation of *Pleurococcus vulgaris* (green coating on tree trunks, roofs, fences, etc.). To inoculate mix a little of the covering from a linden tree with water and pour over the plates. Pour off the excess water. Small green colonies appeared after 3 weeks. Münter used medium 26 to study the availability of carbon source for Actinomyces.

Variants: Münter solidified medium 53 by the addition of agar.

Reference: Beijerinck (1898 p. 785), Münter (1913 p. 368).

1416. Pesch's Basal Ammonium Sulphate Agar

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $MgSO_4$	0.5 g.
4. NaCl	0.02 g.
5. $FeSO_4$	trace
6. $Ca_3(PO_4)_2$	trace
7. $(NH_4)_2SO_4$	1.9 g.
8. Agar	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and one of the added nutrients in 1.

(2) Add Na_2CO_3 until the reaction is slightly alkaline.

(3) Dissolve 20.0 g. of agar that has been washed free from nitrogen in (2).

Sterilization: Not specified.

Use: To study nitrogen and carbon utilization by the colon-typhoid group. Used also as an enrichment medium for the paratyphoid group. The author reported that paratyphoid B and colon organisms showed luxuriant growth. Typhoid and paratyphoid A showed only a little growth even after 48 to 72 hours.

Added nutrients: The author added one of the following materials:

Glucose	10.0 g.
Potassium tartrate	10.0 g.
Sodium citrate	10.0 g.

References: Pesch (1921 pp. 98, 100), Klimmer (1923 p. 217).

1417. Fremlin's Ammonium Sulphate Agar

Constituents:

1. Distilled water	1000.0 cc.
2. $(NH_4)_2SO_4$	1.0 g.
3. Potassium phosphate	1.0 g.

4. $MgCO_3$ (1.0%)	1.0 g.
5. Agar	15.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add 5 and boil.

(3) Tube.

(4) Add 1.0% sterile $MgCO_3$ to sterile (3).

Sterilization: Method of sterilization of (3) or $MgCO_3$ not given.

Use: Isolation of nitroso bacteria and to study nitrogen oxidation by them. Author reported that colonies appeared, when eye was nearly on the level with the colony, as a colorless, ground glass mass 1.0 mm. or more in diameter. Colonies in the depth of medium appeared as points to the naked eye.

Variants: Gage prepared the medium as follows:

(1) Wash agar agar ten times in water to remove all nitrogenous material.

(2) Dissolve 1.0 g. $(NH_4)_2SO_4$, 1.0 g. potassium phosphate and 15.0 g. of (1) in 1000.0 cc. of nitrite free water.

(3) Dissolve 1.0 g. $MgCO_3$ in 100.0 g. nitrite free water.

(4) Sterilize (2) and (3) separately.

(5) Mix (2) and (3) under aseptic conditions.

References: Fremlin (1903 p. 375), Gage (1910 p. 15).

1418. Nencki, Sieber and Wyznikiewicz's Ammonium Sulphate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	10.0 to 15.0 g.
3. K_2HPO_4	0.5 g.
4. Na_2CO_3	1.0 g.
5. $(NH_4)_2SO_4$	2.5 g.
6. NaCl	5.0 to 10.0 g.

Preparation:

(1) Soak 10.0 to 15.0 g. agar with 2 or 3 times the volume of distilled water.

(2) Dissolve the agar in 1000.0 cc. of hot water.

(3) Add 3, 4, 5 and 6 to (2).

(4) Filter.

Sterilization: Sterilize in the autoclave.

Use: Study cause of cattle plague. Author reported that medium when inoculated with cattle plague containing material after two days showed 1–3μ round cells.

Reference: Nencki, Sieber and Wyznikiewicz (1898 p. 530).

1419. Gowda's Ammonium Sulphate Agar

Constituents:

1. Conductivity water.
2. K_2HPO_4.
3. $(NH_4)_2SO_4$.
4. $MgSO_4$.
5. $FeSO_4$.
6. NaCl.
7. Na_2CO_3.
8. Agar.

Preparation:

(1) Soak agar in water for 8 to 10 days.

(2) Dissolve agar in water.

(3) Pour in plates, and while still liquid add the salts as indicated in the table.

(4) The table at top of page 416 gives the amounts of salts used, sterilization, and amount of solution added to each agar plate.

Sterilization: Indicated in table.

Use: To study nitrification.

Reference: Gowda (1924 p. 253).

1420. Heinemann's Ammonium Sulphate Agar

Constituents:

1. Distilled water	1000.0 cc.
2. $FeSO_4$	0.4 g.
3. $MgSO_4$	0.5 g.
4. K_2HPO_4	1.0 g.
5. NaCl	2.0 g.
6. $(NH_4)_2SO_4$	1.0 g.
7. Agar	20.0 g.
8. $CaCO_3$	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Dissolve 20.0 g. of agar in (1).

(3) Add 10.0 g. precipitated $CaCO_3$ to (2).

(4) Shake well.

(5) Tube.

Sterilization: Sterilize in the autoclave.

Use: To study nitrification.

Reference: Heinemann (1922 p. 38).

1421. Committee S. A. B. Basal Ammonium Phosphate Agar

Constituents:

1. Water	1000.0 cc.
2. KCl	0.2 g.
3. $NH_4H_2PO_4$	1.0 g.

SOLUTION NUMBER	a	b			c		STERILIZATION	AMOUNT USED PER PLATE	pH WITH 10 CC. WASHED AGAR
	K_2HPO_4	$(NH_4)_2SO_4$	$MgSO_4$	$Fe_2(SO_4)_3$	NaCl	Na_2CO_3			
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*			
3*	1.5	1.5	0.75	0.02	3.0	1.5	a, b, c, separately	1 cc. each of a, b, c	8.6
4	1.5	1.5	0.75	0.02	3.0	$MgCO_3$ excess	a, b, c, separately	1 cc. each of a, b, c	8.8
5	1.5	1.5	0.75	0.02	3.0	$CaCO_3$ excess	a, b, c, separately	1 cc. each of a, b, c	8.2
6	1.5	1.5	0.75	0.02	$CaCO_3$ excess	$CaCO_3$ excess	a, b, c, separately	1 cc. each of a, b, c	
7	1.5	1.5	0.75	0.02	NaCl 3.0	$MgCO_3$ + $CaCO_3$	a, b, c, separately	1 cc. each of a, b, c	8.0
8a	1.5	1.5	0.75	0.02	NaCl 3.0	Na_2CO_3 1.5	a, b, c, sterilized together	3 cc. sterilized mixture	7.6
8b	1.5	1.5	0.75	0.02	NaCl 3.0	$MgCO_3$ excess	a, b, c, sterilized together	3 cc. sterilized mixture	7.6
8c	1.5	1.5	0.75	0.02	NaCl 3.0	$CaCO_3$ excess	a, b, c, sterilized together	3 cc. sterilized mixture	7.6
9	15.0	15.0	7.50	0.20	NaCl	Na_2CO_3	a, b, c, sterilized separately	0.1 cc. each of a, b, c	
10	15.0	15.0	7.50	0.20	NaCl 30.0	Na_2CO_3 15.0	a, b, c, sterilized together	0.3 cc. of the mixture	

The reaction was determined by the color standards of Medalia (1920), (1922).

* This solution suggested by Skinner and Reid (1921).

4. Brom Cresol Purple, (Sat. Solution) .... 2.0 cc.
5. Agar .... 15.0 g.

Preparation:

(1) Dissolve 2, 3 and 5 in 1.

(2) Adjust to pH = 7.0 by the addition of normal NaOH (6.0 cc.).

(3) Dissolve 1.0% of any desired carbohydrate, alcohol, etc., in (2).

(4) Add 2.0 cc. saturated aqueous solution of brom cresol purple to (3).

(5) Tube.

Sterilization: Method not given.

Use: To study fermentation of carbohydrates, alcohols, etc. A yellow coloration indicates the production of acid.

Added nutrients: The committee recommended addition of 1.0% of any desired carbohydrate, alcohol, etc.

Reference: Committee S. A. B. (1922 p. 522).

1422. Beijerinck's Sodium Ammonium Phosphate Agar

Constituents:

1. Distilled water .... 1000.0 cc.
2. Agar
3. NH_4NaHPO_4 (0.2 to 0.5%) .... 2.0 to 5.0 g.
4. KCl (0.05%) .... 0.5 g.
5. $CaCO_3$

Preparation:

(1) Prepare a solution of agar in distilled water. Exact concentration not specified.

(2) Filter and allow to solidify in flasks in thick layers.

(3) Pour distilled water over the solidified agar and allow to stand for several days. Pour off this water and add fresh distilled water from time to time. This process removes all soluble material from the agar.
(4) Add 0.2 to 0.5% NH_4NaHPO_4 and 0.05% KCl to the agar, also precipitated $CaCO_3$ (amount not given).
(5) Boil. (This serves to sterilize.)
(6) Distribute into sterile dishes.

Sterilization: Sterilization is accomplished in step (5) above.

Use: To show nitrite formation by *Amoeba nitrophil*. Author reported that the colonies producing nitrites formed a clear ring around the colonies due to the action of nitrites on $CaCO_3$. Killer cultivated protozoa and other soil forms on a similar medium.

Variants: Killer specified the use of 0.2% $NH_4NaHPO_4 \cdot 4H_2O$ and omitted the $CaCO_3$.

References: Beijerinck (1896 p. 258), Killer (1913 p. 523).

1423. Stutzer's Ammonium Magnesium Phosphate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	10.0 g.
3. K_2HPO_4	1.0 g.
4. NaCl	5.0 g.
5. $FeSO_4$	0.5 g.
6. Ammonium magnesium phosphate	20.0 g.
7. $MgCO_3$	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Mix equal amounts of 6 and 7 in a mortar.
(3) Add about a half teaspoon of (2) to test tubes.
(4) Add 10.0 to 15.0 cc. of melted (1) to each tube.

Sterilization: Method not specified.

Use: To study nitrification by soil forms.

Reference: Stutzer (1901 p. 173).

1424. Corper's Basal Ammonium Phosphate Agar

Constituents:

1. Water, tap	1000.0 cc.
2. Salt (sea salt)	5.0 g.
3. Ammonium phosphate	3.0 g.
4. Agar	30.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1 by boiling.
(2) Neutralize to litmus by the addition of NaOH.
(3) Add one of the added nutrients listed below.

Sterilization: Sterilize in the autoclave.

Use: Cultivation of tubercle bacilli.

Added nutrients: The author added one of the following:

(a) Glycerol 5.0%
(b) Glycerol 5.0%
Beef extract (Liebig's) 0.3%
(c) Glycerol 5.0%
Peptone (Witte) 1.0%
(d) Glycerol 5.0%
Beef extract (Liebig's) 0.3%
Peptone (Witte) 1.0%
(e) Glycerol 5.0%
Add 1.0% defibrinated rabbit blood at 43°C. and inspissate on each of 3 successive days for 90 minutes.
(f) Same as (e) but using laked blood.
(g) Same as (e) but using cow's milk.
(h) Same as (e) but using 10.0% casein.
(i) Same as (e) but using whole egg.
(j) Same as (e) but using egg white.
(k) Glycerol 5.0%
Add 1.0% sodium nucleinate, prepared by neutralizing yeast nucleic acid in warm water by means of Na_2CO_3 solution. Inspissate as in (e).
(l) Glycerol 5.0%
Add a suspension of yeast nuclein sufficient to make a 1.0% suspension. Inspissate as in (e).
(m) Glycerol 5.0%
Add 1.0% of a mush of testes from rabbits. Inspissate as in (e).
(n) Same as (m) but use liver from rabbits instead of testes.
(o) Same as (m) but use brain from rabbits instead of testes.

Reference: Corper (1919 p. 463).

SUBGROUP II-B. SECTION 3

Basal or complete media containing agar with inorganic constituents. Nitrogen supplied as nitrites or nitrates.

A_1. Nitrogen supplied as nitrites.

Winogradsky's Sodium Nitrite Agar (Fraenkel) 1425
Stutzer's Sodium Nitrite Agar 1426
Heinemann's Sodium Nitrite Agar .. 1427

A_2. Nitrogen supplied as nitrates.

Trautwein's Thiosulphate Nitrate Agar 1428
Beijerinck and van Delden's Nitrate Agar 1429
Noyes' Ammonium Nitrate agar ... 1430

1425. Winogradsky's Sodium Nitrite Agar

Constituents:

1. Water	1000.0 cc.
2. $NaNO_2$ (Merck)	2.0 g.
3. Soda (water free)	1.0 g.
4. Potassium phosphate	
5. Agar agar	15.0 g.

Preparation:

(1) Dissolve 2, 3 and 5 in 1.
(2) Dissolve a knife point of potassium phosphate in (1).

Sterilization: Not specified.

Use: To study nitrification by nitrifying organisms. Stutzer and Hartleb used a similar medium for the cultivation of the supposedly foot and mouth disease bacteria.

Variants:

(a) Stutzer and Hartleb used a medium of the following composition:

(1) Water	1000.0 cc.
(2) Agar	20.0 g.
(3) Potassium phosphate	1.0 g.
(4) Na_2CO_3	1.0 g.
(5) $NaNO_2$	2.0 g.

(b) Fraenkel specified the use of 1.0 g. potassium phosphate and used 12.5 g. of agar instead of 15.0 g.

(c) Omeliansky added a trace of potassium phosphate instead of a knife point.

(d) Wimmer specified the use of 0.5 g. KH_2PO_4 instead of a knife point of potassium phosphate.

(e) Smith added 0.05 g. of K_2HPO_4 instead of a knife point of potassium phosphate.

(f) Löhnis added 0.5 g. K_2HPO_4 instead of a knife point of potassium phosphate.

(g) Percival and Harvey used 0.2 g. of K_2HPO_4 instead of a knife point of potassium phosphate.

References: Winogradsky (1896 p. 425), Stutzer and Hartleb (1897 p. 404), Fraenkel (1898 p. 10), Omeliansky (1899 p. 548), Wimmer (1904 p. 139), Smith (1905 p. 199), Löhnis (1913 p. 110), Percival (1920 p. 150), Harvey (1921–22 p. 107).

1426. Stutzer's Sodium Nitrite Agar

Solidify the variant of Winogradsky's Nitrite Solution as given by Stutzer by the addition of agar. (See variant (a) Med. 1425)

1427. Heinemann's Sodium Nitrite Agar

Constituents:

1. Distilled water	1000.0 cc.
2. $MgSO_4$	0.3 g.
3. $FeSO_4$	0.4 g.
4. NaCl	0.5 g.
5. K_2HPO_4	0.5 g.
6. Na_2CO_3 (fused)	1.0 g.
7. $NaNO_2$	1.0 g.
8. Agar	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Dissolve 20.0 g. of agar in (1).

Sterilization: Sterilize in the autoclave.

Use: Oxidation of nitrites with the formation of nitrates.

Reference: Heinemann (1922 p. 39).

1428. Trautwein's Thiosulphate Nitrate Agar

Solidify Trautwein's Thiosulphate Solution (see medium 109) by the addition of 2.0% agar.

1429. Beijerinck and van Delden's Nitrate Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	15.0 g.
3. K_2HPO_4	0.1 g.
4. KNO_3	0.1 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Isolation of *Bacillus oligocarbophilus*

Variants: Groenewege used 0.1% KNO_3 and 0.05% K_2HPO_4 and added one of the following materials:

sucrose
levulose
maltose
mannitol
glucose

sodium propionate
sodium formate
sodium oxalate
sodium butyrate
sodium succinate

lactose
raffinose
sodium acetate
sodium tartrate
sodium malate
sodium lactate

He reported that lactose, sodium acetate, propionate, formate, oxalate or butyrate were not suitable carbon sources and showed no growth. Remaining materials supported growth. Best growth was obtained with sucrose, glucose and levulose.

References: Beijerinck and van Delden (1903 p. 37), Groenewege (1913 p. 24), Löhnis (1913 p. 106).

1430. Noyes' Ammonium Nitrate Agar

Constituents:

1. Water.................... 1000.0 cc.
2. Agar (best).... .... 15.0 g.
3. NH_4NO_3.................. 1.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: Cultivation of soil organisms. The medium was inoculated with soil.

Reference: Noyes (1916 p. 93).

SUBGROUP II-C

Agar Media with One or More Constituents Organic

Key to the Sections of Subgroup II-C

A_1. Chemical composition of all constituents known.
B_1. Nitrogen inorganic, carbon organic
Section 1 (Med. 1431–1495)
B_2. Nitrogen organic
Section 2 (Med. 1496–1529)
A_2. Chemical composition of at least one constituent not definitely known.
B_1. Containing digests.
C_1. Containing a commercial digest.
D_1. All additional constituents of known chemical composition
Section 3 (Med. 1530–1596)
D_2. At least one of the additional constituents of unknown chemical composition.
E_1. Additional unknown constituent of plant or soil origin
Section 4 (Med. 1597–1627)
E_2. Additional unknown constituent of animal origin.
F_1. Not containing extracts or infusions of animal origin..Section 5 (Med. 1628–1660)
F_2. Containing extracts or infusion of animal origin....Section 6 (Med. 1661–2028)
C_2. Not containing commercial digests; containing non-commercial digests.
Section 7 (Med. 2029–2078)
B_2. Not containing digests.
C_1. Containing unknown constituents of plant origin..Section 8 (Med. 2079–2141)
C_2. Containing unknown constituents of animal origin..Section 9 (Med. 2142–2192)
C_3. Containing unknown constituents of ash or soil origin
Section 10 (Med. 2193–2198)

SUBGROUP II-C. SECTION 1

Basal or complete media containing agar, with all other constituents of known chemical composition; nitrogen inorganic, carbon organic.

A_1.* Nitrogen present as free or atmospheric nitrogen only.
B_1. Carbon supplied as carbohydrates.
C_1. Monosaccharides employed.
D_1. Inorganic salts not added.
Milburn's Basal Glucose Agar...... 1431
Zipfel's Basal Glucose Agar........ 1432
Lantzsch's Basal Nitrate Agar..... 1433
D_2. Inorganic salts added.
E_1. Containing salts of monovalent cations, only.
Davis' Glucose Agar............... 1434
Gerlach and Vogel's Glucose Phosphate Agar...................... 1435
Sullivan's Glucose Agar............ 1436
Gage's Glucose Agar............... 1437
E_2. Containing salts of mono and di-valent cations.
Kisch's Basal Glucose Agar........ 1438
Lipman and Brown's Basal Glucose Agar........................... 1439
Beijerinck's Glucose Agar.......... 1440
Heinemann's Glucose Agar......... 1441
Münter's Basal Salt Agar.......... 1442
C_2. Disaccharides employed.
Groenewege's Basal Sucrose Agar.. 1443
Owen's Sucrose Agar............... 1444
Buchanan's Sucrose Agar.......... 1445
Löhnis' Congo Red Sucrose Agar... 1446
C_3. Polysaccharides employed.
Krainsky's Basal Starch Agar...... 1447
Wyant and Tweed's Starch Agar... 1448
Welch's Dextrin Agar.............. 1449
B_2.† Carbon supplied as alcohols.
C_1. Inorganic salts not added.
Hesse's Glycerol Agar.............. 1450
C_2. Inorganic salts added.

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* See page 420 for A_2 and A_3.

† See page 420 for B_3.

D_1. Containing salts of monovalent cations, only.
Beijerinck's Mannitol Agar........ 1451
D_2. Containing salts of monovalent and other cations.
Löhnis' Basal Glycerol Agar....... 1452
Sackett's Mannitol Salt Agar...... 1453
Ashby's Mannitol Agar (Jones).... 1454
Beijerinck's Mannitol Agar (Omeliansky and Ssewerowa)........... 1455
Shunk's Mannitol Agar............ 1456
Krainsky's Mannitol Agar......... 1457
Heinemann's Mannitol Agar....... 1458
B_3.* Carbon supplied other than alcohols or carbohydrates.
Lieske's Acetate Agar.............. 1459
Söhngen's Petroleum Agar......... 1460
A_2. Nitrogen present as ammonium salts.
B_1. Ammonium chloride employed.
C_1. Carbon supplied as carbohydrates.
Söhngen's Manganese Oxide Agar.. 1461
Conn and Breed's Glucose Ammonium Chloride Agar.............. 1462
Fulmer and Grimes' Sucrose Ammonium Chloride Agar.............. 1463
Groenewege's Cellulose Agar....... 1464
C_2. Carbon supplied as organic acids or their salts.
Söhngen's Organic Acid Agar...... 1465
Krainsky's Malate Agar........... 1466
B_2. Ammonium nitrate employed.
Noyes' Starch Ammonium Nitrate Agar.......................... 1467
B_3. Ammonium sulphate employed. Carbon supplied as carbohydrate.
C_1. Containing monosaccharides.
Kellerman and McBeth's Glucose Ammonium Sulphate Agar....... 1468
Higgins' Glucose Nitrate Agar...... 1469
C_2. Containing disaccharides.
Mortensen's Sucrose Ammonium Sulphate Agar.................. 1470
C_3. Containing polysaccharides.
Kellerman and McBeth's Polysaccharide Ammonium Sulphate Agar.......................... 1471
Sanborn's Ammonium Sulphate Cellulose Agar..................... 1472
Vierling's Cellulose Ammonium Sulphate Agar..................... 1473
B_4. Ammonium carbonate employed.
Proskauer and Beck's Glycerol Ammonium Carbonate Agar (Klimmer)........................... 1474
B_5. Ammonia supplied as salts of phosphoric acid.
C_1. Basal media, employed with the addition of other nutrients.
C_2. Complete media.
D_1. Only one source of organic carbon added.
E_1. Carbon supplied as carbohydrates.
Bengis' Glucose Ammonium Phosphate Agar..................... 1475
Ayers and Rupp's Fuchsin Sulphite Agar............................ 1476
Cunningham's Cellulose Ammonium Phosphate Agar................. 1477
E_2. Carbon supplied as organic acids or their salts.
Bengis' Lactate Ammonium Phosphate Agar..................... 1478
Tausz and Peter's Napthenate Ammonium Phosphate Agar......... 1479
Simmons' Citrate Agar............. 1480
D_2. More than one source of organic carbon added.
Dolt's Lactose Ammonium Phosphate Agar..................... 1481
Harrison and van der Leck's Aesculin Ammonium Phosphate Agar.. 1482
B_6. Ammonium salts of organic acids employed.
Bengis' Ammonium Lactate Agar.. 1483
Cohn's Ammonium Tartrate Agar (Klimmer)........................ 1484
Nelson's Ammonium Succinate Agar 1485
Dolt's Lactose Lactate Agar........ 1486
Harrison and van der Leck's Aesculin Lactate Agar............... 1487
Fischer's Nitrate Tartrate Agar.... 1488
A_3. Nitrogen supplied as nitrates (exclusive of (NH_4NO_3).
B_1. Only one source of organic carbon added.
C_1. Organic carbon supplied as carbohydrate.
Sackett's Glucose Nitrate Agar.... 1489
Conn and Breed's Carbohydrate Nitrate Agar....................... 1490
Groenewege's Sucrose Nitrate Agar. 1491
Czapek's Sucrose Nitrate Agar (Conn).......................... 1492
Pinoy's Dextrin Nitrate Agar...... 1493
Giltay's Glucose Nitrate Agar (Giltner)....................... 1494
C_2. Organic carbon supplied as organic acids or their salts.

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* See also B_4, B_5 and B_6.

B_2. More than one source of organic carbon added.

Conn and Breed's Double Sugar Nitrate Agar.......................... 1495

1431. Milburn's Basal Glucose Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	
3. Glucose (5.0%)	50.0 g.

Preparation:

(1) Dissolve 3 and one of the added nutrients in 1.

(2) Solidify by the addition of agar.

Sterilization: Method not given.

Use: To study color formation. The author reported that tyrosine and asparagin gave concentric circles. Leucin and albumin gave poorly defined green and yellowish white rings. Remaining materials gave no rings. Krainsky used a similar medium for the cultivation of actinomyces.

Added nutrients: The author added 2.0% of one of the following:

tyrosine
asparagin
leucine
albumin
alcumin
nuclean
peptone

Variants: Krainsky used 1.0% glucose and added one of the following materials:

KNO_3	0.05%
NH_4Cl	0.05%
Calcium malate	1.0%
Peptone	0.05%
Asparagin	0.05%

References: Milburn (1904 p. 136), Krainsky's (1914 p. 662).

1432. Zipfel's Basal Glucose Agar

Constituents:

1. Water	1000.0 cc.
2. Agar (3.0%)	30.0 g.
3. Glucose (1.0%)	10.0 g.
4. Malic acid	

Preparation:

(1) Dissolve 2, 3 and one of the added nutrients in 1.

(2) Acidify slightly by the addition of malic acid.

Sterilization: Not specified.

Use: Cultivation of nodule bacteria.

Added nutrients: The author added 2.0% of one of the following:

plasmon
tropon
roborat
sanatogen
nutrose

Reference: Zipfel (1911 p. 128).

1433. Lantzsch's Basal Nitrate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar (2.0%)	20.0 g.
3. K_2HPO_4 (0.01%)	0.1 g.
4. $NaNO_3$ (0.01%)	0.1 g.
5. $CaCl_2$ (0.005%)	0.05 g.
6. $MgSO_4$	traces
7. $FeCl_3$	trace

Preparation:

(1) Wash agar shreds in running water for a week.

(2) Soak (1) in distilled water for one day.

(3) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(4) Pour thick plates.

(5) Incubate the inoculated medium in an atmosphere of one of the added nutrients.

Sterilization: Not specified.

Use: Cultivation of *Actinomyces oligocarbophilus* (*Bacillus oligocarbophilus*).

Added nutrients: The author incubated the medium in an atmosphere of acetic acid, butyric acid or acetone. The atmosphere should contain 0.005 g. acetic acid, 0.005 g. acetone or 0.004 g. butyric acid per 100.0 cc. atmosphere.

Reference: Lantzsch (1922 p. 312).

1434. Davis' Glucose Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. NaCl	5.0 g.
4. Glucose	20.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: To study chromogenesis by sporotricha. Author reported that if maltose be substituted for glucose, growth and pigment production was more luxuriant. Using glucose, pigment formation was slight. Growth without glucose about the same as when glucose (or impure glucose) was employed.

Reference: Davis (1915 p. 178).

1435. Gerlach and Vogel's Glucose Phosphate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	20.0 g.
3. Potassium bi-phosphate	2.0 g.
4. Glucose	2.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not given.

Use: Cultivation of Azotobacter (nitrogen assimilators from the soil). Other investigators used different nitrogen fixing organisms.

Variants:

(a) Beijerinck and van Delden used 0.5 g. K_2HPO_4 instead of 2.0 g. of potassium bi-phosphate, and solidified the medium with agar, not specifying the exact amount used. These investigators cultivated chroococcum.

(b) Stoklasa isolated radiobacteria, azotobacter and chroococcum in a medium composed of 1000.0 cc. of Moldau river water, 20.0 g. agar, 20.0 g. glucose and 5.0 g. potassium diphosphate.

(c) Percival used 2.0 g. K_2HPO_4 per liter.

References: Gerlach and Vogel (1902 p. 670), Beijerinck and van Delden (1902 p. 8), Stoklasa (1908 p. 489), Percival (1920 p. 182).

1436. Sullivan's Glucose Agar

Constituents:

1. Water	1000.0 cc.
2. Glucose	20.0 g.
3. Na_3PO_4	5.0 g.
4. K_2HPO_4	5.0 g.
5. Agar	10.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: General culture medium. Author reported that few organisms were capable of growing in this medium.

Reference: Sullivan (1905–06 p. 117).

1437. Gage's Glucose Agar

Constituents:

1. Water (nitrate free)	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. NaCl	1.0 g.
4. Agar	15.0 g.
5. Glucose	1.0 g.

Preparation:

(1) Wash agar thoroly in water until it is free from nitrogen.

(2) Dissolve 2, 3, 15.0 g. of (1) and 1.0 g. of glucose in 1000.0 cc. of nitrite free water.

(3) Distribute into large Erlenmeyer flasks.

Sterilization: Method not given.

Use: To study nitrogen fixation by nitroso bacteria. The author also substituted lactose for glucose.

Reference: Gage (1910 p. 18).

1438. Kisch's Basal Glucose Agar

Medium the same as 129, but solidified by the addition of 2.0% agar.

1439. Lipman and Brown's Basal Glucose Agar

Constituents:

1. Water	1000.0 cc.
2. Glucose	10.0 g.
3. K_2HPO_4	0.5 g.
4. $MgSO_4$	0.2 g.
5. Agar	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and one of the added nutrients in 1.

(2) Add NaOH to neutralize one-half or one-fourth of the acid or add no NaOH.

Sterilization: Method not given.

Use: To determine bacterial count of soils. Other investigators used the basic medium without additions for the cultivation of yeast.

Added nutrients: The authors added 0.05 g. KNO_3, 0.04 g. $(NH_4)_2SO_4$ or 0.05 g. peptone to the basic solution.

Variants:

(a) Dombrowski cultivated milk yeast in a medium of the following composition:

1. Water	1000.0 cc.
2. $MgSO_4$ (0.1%)	1.0 g.
3. K_2HPO_4 (0.2%)	2.0 g.
4. Dextrose (5.0%)	50.0 g.
5. Agar (1.5%)	15.0 g.

(b) Beijerinck cultivated *Odium lactis* in a medium composed of the following constituents:

1. Water	1000.0 cc.
2. Agar	20.0 g.

3. Glucose	50.0 g.
4. KH_2PO_4	0.5 g.
5. $MgSO_4$	0.2 g.

(c) Beijerinck also used the following medium:

1. Water	1000.0 g.
2. Glucose	100.0 g.
3. KH_2PO_4	1.0 g.
4. $MgSO_4$	1.0 g.
5. Agar	20.0 g.

(d) Brown added a trace of Fe_2SO_4 to the basic medium and added 0.05 g. peptone. This medium was modified as follows:

(1) Substitute 0.05 g. urea for peptone (Brown urea agar).

(2) Substitute 0.05 g. asparagin for peptone (Brown asparagin agar).

(3) Substitute 0.1 g. casein for peptone (Brown casein agar A).

(4) Substitute 0.1 g. albumin for peptone (Brown albumin agar A).

(5) Omit the peptone from (1) and substitute 10.0 g. of albumin for the peptone (Brown albumin agar B).

(6) Substitute 20.0 cc. of extract of humus (method of preparation not given) for peptone (Brown Artificial humus agar A).

(7) Substitute 10.0 cc. of extract of humus (method of preparation not given) for peptone (Brown Artificial humus agar B).

(8) Substitute 20.0 cc. of humus extract (method of preparation not given) for peptone and add 6.0 g. K_2HPO_4 (Brown Artificial humus agar C). Reaction is 0.5% acid.

(9) Substitute 10.0 cc. of humus extract (method of preparation not given) for peptone and add 3.0 g. K_2HPO_4 (Brown Artificial humus agar D). Reaction is 0.5% acid.

(10) Same as (9) but omit the dextrose from the agar. Reaction is 0.5% acid (Brown Artificial humus agar E).

(11) Substitute 10.0 cc. of humus extract neutralized with HCl for peptone (Brown Artificial humus agar F).

(12) Substitute 25.0 cc. of humus extract neutralized with N/1 HCl for peptone (Brown Artificial humus agar G).

(13) Substitute 0.05 g. casein for peptone (Brown casein agar B).

(14) Substitute 0.05 g. albumin for peptone (Brown albumin agar C).

(15) Substitute 0.5 g. albumin for peptone (Brown albumin agar D).

(16) Substitute 1.0 g. albumin for peptone (Brown albumin agar E).

(17) Substitute 0.5 g. casein for peptone (Brown casein agar C).

(e) Greaves studied the bacterial count of soil on a medium composed of the following constituents:

1. Distilled water	1000.0 cc.
2. Dextrose	10.0 g.
3. K_2HPO_4	0.5 g.
4. $MgSO_4$	0.2 g.
5. Agar (powdered)	20.0 g.

References: Lipman and Brown (1910 p. 447), Dombrowski (1910 p. 380), Beijerinck (1911 pp. 164, 165), Brown (1913 p. 498), Greaves (1914 p. 447).

1440. Beijerinck's Glucose Agar

Same as medium 185, but solidified by the addition of 2.0% agar.

1441. Heinemann's Glucose Agar

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4	1.0 g.
3. $MgSO_4$	0.5 g.
4. NaCl	0.01 g.
5. $FeSO_4$	0.01 g.
6. $MnSO_4$	0.01 g.
7. Glucose	20.0 g.
8. Agar	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Dissolve 20.0 g. of agar in (1).

Sterilization: Sterilize in the autoclave.

Use: To study the assimilation of atmospheric nitrogen.

Reference: Heinemann (1922 p. 39).

1442. Münter's Basal Salt Agar

Same as medium 164, but solidified by the addition of 1.2% agar. Also solidify medium 140 by the addition of 1.2% agar.

1443. Groenewege's Basal Sucrose Agar

Constituents:

1. Water (tap)	1000.0 cc.
2. Agar to solidify	
3. Sucrose	20.0 g.
4. K_2HPO_4	0.5 g.

Preparation: (1) Dissolve 3, 4 and one of the added nutrients in tap water agar (preparation not given).

Sterilization: Not specified.

Use: Cultivation of *Phytobacter lycopersicum* n. sp. causing tomato rot. Author reported growth with all nitrogen sources employed.

Added nutrients: The author added one of the following nitrogen compounds as a nitrogen source:

KNO_3
$(NH_4)_2SO_4$
sodium ammonium tartrate
ammonium citrate
ammonium succinate
ammonium acetate
ammonium lactate
ammonium malate
asparagin

Reference: Groenewege (1913 p. 25).

1444. Owen's Sucrose Agar

Constituents:

1. Water	1000.0 cc.
2. Agar (2.0%)	20.0 g.
3. Sucrose (Second 80°) (10.0%)	100.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Method not given.

Use: To determine bacterial count in cane sugar products.

Reference: Owen (1914 p. 338).

1445. Buchanan's Sucrose Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	10.0 g.
3. Sucrose	20.0 g.
4. KH_2PO_4	0.2 g.
5. $MgSO_4$	0.01 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Pour sterile (1) into sterile plates.

Sterilization: Method not specified.

Use: To study the production of gum by *B. radicicola*, isolated from a variety of hosts. Various authors used the same or similar media for a variety of purposes.

Variants:

(a) Peklo cultivated plant actinomyces on a medium solidified with agar containing 0.5% KH_2PO_4, 0.2% $MgSO_4$ and did not specify the use of 1.0% agar as did Buchanan.

(b) Tanner, citing Moore, cultivated *Ps. radicicola* on a medium containing 0.1% K_2HPO_4, 0.05% $MgSO_4$, 1.0% sucrose and 1.0% agar.

(c) Shunk dissolved 1.0 g. KH_2PO_4, 0.5 g. $MgSO_4$, 10.0 g. sucrose and 10.0 g. or 15.0 g. agar in tap water. This medium was employed for the isolation of nodule bacteria. He reported that if mannitol be substituted for sucrose, the medium could be used to maintain cultures.

References: Buchanan (1909 p. 381), Peklo (1910 p. 470), Tanner (1919 p. 50), Shunk (1921 p. 241).

1446. Löhnis' Congo Red Sucrose Agar

Bonstituents:

1. Water	1000.0 cc.
2. Sucrose	10.0 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4$	0.2 g.
5. Agar	15.0 g.
6. Congo red	0.1 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Differentiation of *B. tumefaciens* and *B. radicicola*. Author reported that *B. tumefaciens* absorbed the congo red giving red colonies while *B. radicicola* formed white colonies.

Variants: (a) Giltner specified the use of washed agar.

References: Löhnis (1913 p. 112), Giltner (1921 p. 377).

1447. Krainsky's Basal Starch Agar

Constituents:

1. Water	1000.0 cc.
2. Agar to solidify	
3. Starch (1.0%)	10.0 g.

Preparation:

(1) Dissolve 3 and one of the added nutrients in 1.

(2) Solidify (1) by the addition of agar.

Sterilization: Not given.

Use: Cultivation of Actinomyces.

Added nutrients: The author added 0.05% of one of the following:

KNO_2
KNO_3
NH_4Cl
Peptone
Asparagin

Variants: Noyes dissolved 1.0 or 2.0 g. of starch and 15.0 g. agar in 1000.0 cc. of water, and used the medium for soil organisms.

References: Krainsky (1914 p. 662), Noyes (1916 pp. 93, 94).

1448. Wyant and Tweed's Starch Agar

Constituents:

1. Water	1000.0 cc.
2. Starch (potato)	10.0 g.
3. Agar	10.0 g.
4. K_2HPO_4	0.2 g.
5. $MgSO_4$	0.2 g.
6. K_2CO_3	0.4 g.
7. $CaCl_2$	0.02 g.
8. $Fe_2(SO_4)_3$	0.02 g.
9. NaCl	0.02 g.

Preparation:

(1) Prepare a starch solution by suspending 10.0 g. potato starch in a little cold water. Then add 500.0 cc. boiling water. Concentrate to 500.0 cc.

(2) Dissolve 3, 4, 5, 6, 7, 8 and 9 in 500.0 cc. water by boiling.

(3) Replace amount of water lost by addition of distilled water.

(4) Add (1) to (3).

Sterilization: Not specified.

Use: Cultivation of organisms causing flat sour in peas.

Reference: Wyant and Tweed (1923 p. 12).

1449. Welch's Dextrin Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Dextrin	10.0 g.
3. K_2HPO_4	2.0 g.
4. $MgSO_4$	0.2 g.
5. $CaCO_3$	0.2 g.
6. Agar	10.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Isolation of amoeba.

Reference: Welch (1917 p. 5) taken from (1917 p. 396).

1450. Hesse's Glycerol Agar

Constituents:

1. Distilled water	960.0 cc.
2. Glycerol	30.0 g.
3. Agar	10.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Filter and distribute in 20.0 cc. lots in 50.0 cc. Jena glass flasks.

(3) Melt the sterile agar and cool to 40°C.

(4) Place a portion of the sputum upon a piece of litmus paper.

(5) Add 1/10 N KOH to the agar until the same shade of blue is given to litmus paper as is given by the sputum. One may take a series of 6 flasks containing 20.0 cc. of distilled water and add 0.0, 0.2, 0.5, 1.0, 2.0 and 5.0 cc. respective of 1/10 N KOH to the flasks and in this manner determine the proper amount of KOH to add to the agar to give the desired reaction.

(6) Pour the agar into sterile Petri dishes.

Sterilization: Sterilize for 3 hours in streaming steam or autoclave for 1 hour under two atmospheres of pressure.

Use: Isolation of tubercle bacilli from sputum. Author reported that the colonies were seen under the microscope after 1 or 2 days. If accompanying bacteria are to be studied add a small amount of Nährstoff Heyden or 0.1% grape sugar.

References: Hesse (1904 p. 386), Abel (1912 p. 95).

1451. Beijerinck's Mannitol Agar

Same as medium 121, but solidified by the addition of 2.0% agar.

1452. Löhuis' Basal Glycerol Agar

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	0.5 g.
3. $MgSO_4$	0.02 g.
4. NaCl	0.02 g.
5. Fe_2Cl_6	trace
6. Agar (1.5%)	15.0 g.
7. Glycerol (1.0%)	10.0 g.

Sterilization:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Dissolve 1.0% glycerol and 0.1% of one of the added nutrients to (1).
(3) Distribute in 10.0 cc. quantities in 50.0 cc. Erlenmeyer flasks.

Sterilization: Not specified.

Use: To study assimilation of amide, ammonia and nitrate. The author suggested that any desired carbohydrate, alcohol, etc., might replace the glycerol.

Added nutrients: The author suggested the addition of 0.1% of one of the following:

Urea
Uric acid
Sodium hippurate
Asparagin
$(NH_4)_2SO_4$
Ammonium acetate
Ammonium butyrate
Ammonium lactate
$NaNO_3$

Reference: Löhnis (1913 p. 99).

1453. Sackett's Mannitol Salt Agar

Constituents:

1. Distilled water	1000.0	cc.
2. Na_2SO_4	9.0668	g.
3. NaCl	20.2621	g.
4. Na_2CO_3	2.0118	g.
5. $MgSO_4$	1.7475	g.
6. Mannitol	15.0	g.
7. $CaSO_4$	9.4457	g.
8. $NaNO_3$	13.5319	g.
9. Agar	15.0	g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.
(2) Dissolve 15.0 g. of agar in 1000.0 cc. of (1).
(3) Tube.

Sterilization: Autoclave at 120°C. for 5 minutes.

Use: To study pigment production by *Azotobacter chroococcum.* The author reported generally a brownish pigment was produced. No pigment was produced if mannitol be omitted.

Variants: The author gave the following variants:

(a) Omitted the $CaSO_4$ and $NaNO_3$ and added 2.8589 g. Na_2CO_3.
(b) Omitted the NaCl.
(c) Omitted the $NaNO_3$.
(d) Omitted the Na_2SO_4.
(e) Omitted the $MgSO_4$.
(f) Omitted the K_2SO_4.
(g) Diluted any of the above media with 2 or 3 volumes of water.

Reference: Sackett (1912 p. 109).

1454. Ashby's Mannitol Agar (Jones)

Same as medium 170, but solidified by the addition of 1.5% agar.

1455. Beijerinck's Mannitol Agar (Omeliansky and Ssewerowa)

Same as medium 148, but solidified by the addition of 1.5% agar.

Murray gave a modification of the same medium by solidifying medium 149 by the addition of 1.5% agar.

1456. Shunk's Mannitol Agar

Variant (c) of medium 1445 solidified with agar.

1457. Krainsky's Mannitol Agar

Same as medium 188, but solidified by the addition of 2.0% agar.

1458. Heinemann's Mannitol Agar

Constituents:

1. Distilled water	1000.0	cc.
2. K_2HPO_4	0.2	g.
3. $CaCl_2$	0.02	g.
4. $MgSO_4$	0.2	g.
5. Fe_2Cl_6	0.01	g.
6. Mannitol	15.0	g.
7. Agar	20.0	g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Add NaOH solution until the reaction is neutral to phenolphthalein.
(3) Dissolve 20.0 g. of agar in (2).

Sterilization: Sterilize in the autoclave.

Use: Cultivation of cultures capable of assimilating atmospheric nitrogen.

Reference: Heinemann (1922 p. 39).

1459. Lieske's Acetate Agar

Constituents:

1. Distilled water	1000.0	cc.
2. Agar	10.0	g.
3. Manganese acetate	0.1	g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: Isolation of *Leptothrix ochracea.* Inoculate the agar plates when the surface is still moist.

Reference: Lieske (1919 p. 418).

1460. Söhngen's Petroleum Agar

Constituents:

1. Distilled water	1000.0	cc.
2. Bipotassium phosphate	0.5	g.

3. $MgSO_4$	0.5	g.
4. Agar	20.0	g.
5. Petroleum		

Preparation:

(1) Dissolve 2, 3 and 20.0 g. of washed agar in 1.

(2) Adjustment of reaction not given.

(3) Pour in Petri dishes.

(4) Invert the Petri dishes, and place a little petroleum in a flat container, on the lid inside the petri dish. This places the surface of the medium directly opposite the surface of the petroleum.

Sterilization: Method not given.

Use: Cultivation of petroleum oxidizers, soil forms *Mycobacterium hyalinum Mycobacterium phlei, Mycobacterium lacticola, Mycobacterium rubrum, Mycobacterium album.*

Reference: Söhngen (1913 p. 598).

1461. Söhngen's Manganese Oxide Agar

Constituents:

1. Water agar	1000.0	cc.
2. Glucose	5.0	g.
3. K_2HPO_4	0.05	g.
4. NH_4Cl	0.05	g.
5. Manganese oxide		

Preparation:

(1) Dissolve 2, 3 and 4 in water agar (composition not given).

(2) Add manganese oxide until a black color is formed.

Sterilization: Method not given.

Use: Cultivation of manganese bacteria. Author reported that a clearing of the medium near the colony indicated the decomposition of the manganese oxide. *Acetobacter melanogenum, B. lactis aërogenes, B. herbicola, B. aceti, B. rancens* and others decomposed manganese oxide.

Reference: Söhngen (1914 p. 554).

1462. Conn and Breed's Glucose Ammonium Chloride Agar

Constituents:

1. Water	1000.0	cc.
2. Agar	15.0	g.
3. Glucose	10.0	g.
4. KNO_3	1.0	g.
5. $CaCl_2$	0.5	g.
6. K_2HPO_4	0.5	g.
7. NH_4Cl	2.0	g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Test for reduction of nitrate.

Reference: Conn and Breed (1919 p. 278).

1463. Fulmer and Grimes' Sucrose Ammonium Chloride Agar

Constituents:

1. Distilled water	1000.0	cc.
2. NH_4Cl	1.88	g.
3. K_2HPO_4	1.0	g.
4. $CaCl_2$	1.0	g.
5. Sucrose	50.0	g.
6. Agar	15.0	g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Wash agar several times in distilled water.

(3) Add agar to (1), heat in autoclave at 15 pounds pressure for 30 minutes.

(4) Filter thru absorbent cotton and tube.

Sterilization: Sterilize in live steam for 30 minutes on two successive days.

Use: Cultivation of yeast, *Saccharomyces cerevisiae* and *Torula sphaerica.*

Variants: The authors omitted the $CaCl_2$. Other experiments were reported omitting the sucrose.

Reference: Fulmer and Grimes (1923 p. 586).

1464. Groenewege's Cellulose Agar

Constituents:

1. Water agar	100.0	cc.
2. Cellulose	2.0	g.
3. NH_4Cl	0.1	g.
4. K_2HPO_4	0.05	g.

Preparation: (1) Dissolve 2, 3 and 4 in tap water agar (composition not given).

Sterilization: Not specified.

Use: Cultivation of *Phytobacter lycopersicum* causing tomato rot. Author reported that organism did not utilize cellulose. A slight growth appeared after several weeks, however, due possibly to a trace of starch in the filter paper used as cellulose source.

Reference: Groenewege (1913 p. 25).

1465. Söhngen's Organic Acid Agar

Constituents:

1. Distilled water	1000.0	cc.
2. Agar	20.0	g.
3. NH_4Cl	0.5	g.

4. $MgSO_4$	0.5 g.
5. Bipotassium phosphate	0.5 g.
6. Sodium butyrate	10.0 g.
7. Chemical elements	

Preparation:

(1) Dissolve 2, 3, 4 and 6 in 1.

(2) Add several elements. (Elements or compounds not given.)

Sterilization: Not given.

Use: Cultivation of Mycobacteria. Author reported that sulphur, potassium, phosphorous nitrogen and carbon were necessary for growth of mycobacteria. The author also studied the production of manganese oxide by bacteria on a similar medium.

Variants: The author reported that when manganese oxide was produced on the following medium a dark brown or black colony was formed:

1. Water agar	1000.0 cc.
2. NH_4Cl	0.5 g.
3. K_2HPO_4	0.5 g.
4. Calcium malate or calcium gluconate	20.0 g.
5. Manganese sulphate	10.0 g.

Reference: Söhngen (1913 p. 605), (1914 p. 546).

1466. Krainsky's Malate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar to solidify	
3. NH_4Cl (0.05%)	0.5 g.
4. Calcium malate (1.0%)	10.0 g.

Preparation:

(1) Dissolve 0.05% NH_4Cl and 1.0% calcium malate (or the author substituted 1.0% calcium nitrate) in water.

(2) Solidify (1) by the addition of agar.

Sterilization: Method not given.

Use: Cultivation of actinomyces.

Reference: Krainsky (1914 p. 662).

1467. Noyes' Starch Ammonium Nitrate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar (best)	15.0 g.
3. NH_4NO_3	1.0 g.
4. Starch	1.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Method not given.

Use: Cultivation of soil forms.

Reference: Noyes (1916 pp. 93, 94).

1468. Kellerman and McBeth's Glucose Ammonium Sulphate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	10.0 g.
3. Glucose	10.0 g.
4. K_2HPO_4	1.0 g.
5. $MgSO_4$	1.0 g.
6. NaCl	1.0 g.
7. $(NH_4)_2SO_4$	2.0 g.
8. $CaCO_3$	2.0 g.

Preparation:

(1) Dissolve 4, 5, 6, 7 and 8 in 1000.0 cc. of water.

(2) Mix 500.0 cc. of (1) and 500.0 cc. of water.

(3) Dissolve 10.0 g. agar and 10.0 g. glucose in (2).

Sterilization: Not given.

Use: Cultivation of *Bacillus flavigena, Bacillus amylolyticus, Bacillus rossica n. sp.*

Reference: Kellerman and McBeth (1912 p. 487).

1469. Higgins' Glucose Nitrate Agar

Same as medium 270, but solidified by the addition of agar.

1470. Mortensen's Sucrose Ammonium Sulphate Agar

Same as medium 207, but solidified by the addition of 2.0% agar.

1471. Kellerman and McBeth's Polysaccharide Ammonium Sulphate Agar

Constituents:

1. Water	1000.0 cc.
2. Cellulose	15.0 g.
3. Agar	10.0 g.
4. K_2HPO_4	1.0 g.
5. $MgSO_4$	1.0 g.
6. NaCl	1.0 g.
7. $(NH_4)_2SO_4$	2.0 g.
8. $CaCO_3$	2.0 g.

Preparation:

(1) Prepare a liter of dilute ammonium hydroxide solution by adding three parts water to ten parts ammonium hydroxide, sp. gr. 0.90.

(2) Add a slight excess of copper carbonate.
(3) Shake vigorously.
(4) Allow to stand over night and then siphon off the supernatant solution.
(5) Add 15.0 g. of unwashed sheet filter paper.
(6) Shake occasionally until the paper is dissolved.
(7) Dilute to 10 liters and add slowly a one to five solution of hydrochloric acid with vigorous shaking until the precipitation of the cellulose is complete.
(8) Dilute to 20 liters, allow the cellulose to settle and decant the supernatant liquid.
(9) Wash by repeated changes of water, adding HCl each time until the copper color disappears. Then wash with water alone until the solution is free from chlorine.
(10) Allow to settle several days and decant as much of the clear solution as possible.
(11) If the cellulose percentage is too low centrifuge a portion of the fluid to bring the cellulose content up to one per cent.
(12) Dissolve 4, 5, 6, 7 and 8 in 1000.0 cc. of tap water.
(13) Mix 500.0 cc. of (11), 500.0 cc. of (12).
(14) Dissolve 10.0 g. of agar in (13).

Sterilization: Method not given.

Use: Cultivation of organisms fermenting cellulose.

Variants:

(a) The author prepared a similar medium using potato starch instead of cellulose as the polysaccharide. The medium was prepared as follows:
  (1) To 800.0 cc. of boiling water add 10.0 g. of potato starch suspended in cold water.
  (2) Evaporate by boiling to 500.0 cc.
  (3) Dissolve 1.0 g. K_2HPO_4, 1.0 g. $MgSO_4$, 1.0 g. NaCl, 2.0 g. $(NH_4)_2SO_4$, and 2.0 g. $CaCO_3$ in 1000.0 cc. of water.
  (4) Mix 500.0 cc. (2) with 500.0 cc. (3).
  (5) Dissolve 10.0 g. of agar in (4).
  (6) Sterilization not specified.

(b) Tanner used one-half the amounts of salts and dissolved them in 500.0 cc. of water instead of 1000.0 cc. as indicated by Kellerman and McBeth. Tanner then mixed equal parts salt solution on cellulose solution.

(c) Tanner prepared starch agar by dissolving one-half the quantity of salts given in variant (a), step (3), in 500.0 cc. tap water. Then Tanner mixed equal parts of salt solution and starch water and proceeded as in variant (a).

(d) Giltner prepared a starch agar as follows:
  (1) Dissolve 1.0 g. K_2HPO_4, 1.0 g. $MgSO_4 \cdot 7H_2O$, 1.0 g. NaCl, 2.0 g. $(NH_4)_2SO_4$, and 2.0 g. $CaCO_3$ in 1000.0 cc. of water.
  (2) Add 10.0 g. washed agar.
  (3) Weigh.
  (4) Boil over a free flame until the solution of the agar is complete.
  (5) Weigh and make up the loss in weight with boiling water.
  (6) Make a smooth paste of 10.0 g. of potato starch or other starch in a little cold water.
  (7) Add 800.0 cc. of boiling water to (6).
  (8) Concentrate to 500.0 cc. by boiling.
  (9) Mix (5) and (8) and boil a few minutes.
  (10) Strain thru two thicknesses of cheese cloth.
  (11) Add 1.5% china blue rosolic acid for indicator.
  (12) Tube, taking care to keep the $CaCO_3$ well mixed with the media.
  (13) Sterilize (method not given).

(e) Khouvine prepared a cellulose in the same manner as Kellerman and McBeth and dissolved 20.0 g. agar in 1000.0 cc. of water by heating. The inorganic salts, as used by Kellerman and McBeth, were dissolved in the agar solution. Five hundred cubic centimeters of the cellulose solution was then added to the agar solution, mixed well, tubed and sterilized at 110° for 15 minutes.

(f) Khouvine prepared a starch agar in the following manner:
  (1) Prepare a paste with 10.0 g. of potato starch in a little cold water.
  (2) Add (1) to 800.0 cc. of boiling water.

(3) Evaporate to 500.0 cc.
(4) Dissolve 20.0 g. agar in 1000.0 cc. water.
(5) Dissolve 1.0 g. K_2HPO_4, 1.0 g. $MgSO_4$, 1.0 g. NaCl, 2.0 g. $(NH_4)_2SO_4$ and 2.0 g. $CaCO_3$ in (4).
(6) Mix (3) and (5).
(7) If deep agar tubes are to be prepared it is well to filter until clear. Filtering is carried out in the autoclave.
(8) Distribute in tubes and sterilize in the autoclave at 100°C. for 15 minutes.
(9) Medium may be poured into sterile petri dishes.

References: Kellerman and McBeth (1912 p. 487), Tanner (1919 pp. 61, 62), Giltner (1921 p. 371), Khouvine (1923 pp. 714, 715).

1472. Sanborn's Ammonium Sulphate Cellulose Agar

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $MgSO_4$	1.0 g.
4. Na_2CO_3	1.0 g.
5. $(NH_4)_2SO_4$	2.0 g.
6. Cotton	30.0 g.
7. Agar	2.5 g.
8. CR indicator (1.0%)	

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 500.0 cc. of distilled water.
(2) Prepare a 0.5% agar solution with the remaining 500.0 cc. distilled water.
(3) Mix (1) and (2).
(4) Cut 30.0 g. of cotton (chemically untreated) into small fragments.
(5) Add 1.0% CR indicator with constant stirring. (CR indicator prepared by mixing equal parts of 0.5% aqueous solution of china blue with 1.0% solution of rosolic acid in 95.0% alcohol.)
(6) Mix well to insure an even distribution of cotton and pour into petri dishes.

Sterilization: The medium was autoclaved after it had been added to the dishes. The medium is colored red, pH-8.4.

Use: To show fermentation of cellulose by *Cellulomonas folia* in the presence of *Act. colorata*, *Azotobacter*, *B. subtilis*, *B. mycoids* and *B. cereus*.

Reference: Sanborn (1926 p. 353).

1473. Vierling's Cellulose Ammonium Sulphate Agar

Constituents:

1. Water	1000.0 cc
2. K_2HPO_4	1.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.1 g.
5. $FeCl_3$	trace
6. NaCl	trace
7. Agar	20.0 g.
8. Cellulose (Merck)	10.0 g.
9. Potassium stearate	1.0 g.
10. $(NH_4)_2SO_4$	5.0 g.
11. $CaCO_3$	1.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 in 1.

Sterilization: Method not given.

Use: Decomposition of cellulose by Mycobacteria. Incubate under a glass bell jar. Author reported that the filter paper showed no signs of being attacked. Growth occurred, however. The agar became turbid. After incubation no clearing occurred around the colonies. Cellulose was not decomposed.

Reference: Vierling (1920 p. 206).

1474. Proskauer and Beck's Glycerol Ammonium Carbonate Agar (Klimmer)

Constituents:

1. Water	1000.0 cc.
2. Glycerol	15.0 g.
3. $(NH_4)_2CO_3$	3.5 g.
4. $MgSO_4$ (crystalline)	2.5 g.
5. KH_2PO_4	1.3 g.
6. Agar	15.0 to 30.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Method not given.

Use: Cultivation of tubercle bacilli.

Reference: Klimmer (1923 p. 172).

1475. Bengis' Glucose Ammonium Phosphate Agar

Constituents:

1. Distilled water	100.0 cc.
2. Agar (powdered)	2.0 g.
3. Glucose	2.0 g.
4. $(NH_4)_2HPO_4$	0.1 g.

5. $MgSO_4$	0.5 g.
6. $CaCO_3$	1.0 g.

Preparation:

(1) Dissolve 2 in 1.

(2) Add 3, 4, 5 and 6 to (1).

(3) Filter to incubating flasks.

Sterilization: Sterilize in autoclave.

Use: To cultivate large quantities of *B. coli*.

Reference: Bengis (1916 p. 392).

1476. Ayers and Rupp's Fuchsin Sulphite Agar

Constituents:

1 Distilled water	1000.0 cc.
2. Sodium ammonium phosphate	4.0 g.
3. KH_2PO_4	2.0 g.
4. Lactose	
5. Agar	30.0 g.
6. Fuchsin (1.0% alcoholic basic soln.)	5.0 cc.
7. Na_2SO_4 (5.0% aque. solution)	5.0 cc.

Preparation:

(1) Dissolve 2, 3 and 4 in 500.0 cc. of 1.

(2) Dissolve agar in 500.0 cc. distilled water and filter.

(3) Mix equal parts (1) and (2) and flask in 100.0 cc. lots.

(4) Prepare 1.0% alcoholic solution of basic fuchsin.

(5) Prepare 5.0% H_2O solution of sodium sulfite.

(6) To every 100.0 cc. of sterile (3) while hottest, at time of plating add 0.5 cc. of (4) and 0.5 cc. of (5) freshly prepared.

(7) Mix thoroughly.

Sterilization: Method of sterilization of (3) not given.

Use: Enumeration of colon-aerogenes group. Author reported the members of the colon-aerogenes produced medium sized red colonies with a ring around the colony. Other colonies were pink. Purified litmus or brom cresol purple were not as well suited as indicators as was the fuchsin sulphite mixture.

Variants: Harvey prepared a similar medium as follows:

(1) Dissolve 2.0 g. sodium ammonium phosphate, 1.0 g. KH_2PO_4, 5.0 g. lactose, 15.0 g. agar in 1000.0 cc. distilled water.

(2) Add to this mixture at 90°C.: 1% alcoholic basic fuchsin 0.5; freshly prepared 5% sodium sulphite 0.5.

References: Ayers and Rupp (1918 pp. 433, 434), Harvey (1921–22 p. 103).

1477. Cunningham's Cellulose Ammonium Phosphate Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar (1.5%)	15.0 g.
3. Cellulose	
4. $MgNH_4PO_4$ (0.05%)	0.5 g.
5. K_2HPO_4 (0.05%)	0.5 g.

Preparation:

(1) Wash 15.0 g. of agar by immersing it in distilled water for about a week. Change the water each day.

(2) Add sufficient distilled water to make a 1.5% agar solution.

(3) Steam (2) for one hour.

(4) Dissolve 0.05% $Mg(NH_4)PO_4$ and 0.05% K_2HPO_4 in (3).

(5) Tube in 4 to 8.0 cc. quantities.

(6) Melt the sterile agar and pour an 8.0 cc. tube into a petri dish.

(7) Place a sterile disc of filter paper on the agar following inoculation and gently press it down. Then pour 4.0 cc. of sterile melted agar over the filter paper.

Sterilization: Sterilize (5) intermittently in steam. Sterilize, in the autoclave, pieces of filter paper cut to fit a petri dish and test tubes.

Use: Decomposition of cellulose by soil forms.

Reference: Cunningham (1924 p. 140).

1478. Bengis' Lactate Ammonium Phosphate Agar

Constituents:

1. Distilled water	100.0 cc.
2. $(NH_4)_2HPO_4$	0.2 g.
3. Calcium lactate	1.0 g.
4. Agar (powdered)	3.0 g.

Preparation:

(1) Dissolve 4 in 1.

(2) Add and dissolve 2 and 3 in (1).

(3) Filter into incubating flasks.

Sterilization: Sterilize in a Bramhall-Dean autoclave.

Use: To cultivate large quantities of *B. coli*. Author reported slight growth.

Reference: Bengis (1916 p. 392).

1479. Tausz and Peter's Napthenate Ammonium Phosphate Agar

Constituents:

1. Water	1000.0 cc.
2. $MgNH_4PO_4$ (0.1%)	1.0 g.
3. $CaSO_4$ (0.01%)	0.1 g.
4. K_2HPO_4 (0.08%)	0.8 g.
5. $FeCl_3$	trace
6. KI	trace
7. Agar	15.0 g.
8. Sodium salt of napthenic acid	10.0 g.

Preparation:

(1) Cut agar into small pieces, mix with water and allow to stand (time not specified).
(2) Wash with flowing water on a filter for several days.
(3) Dissolve 2, 3, 4, 5, 6 and (2) in 1.
(4) Add 1.0 g. of the sodium salt of napthenic acid to each 100.0 cc. of (3).

Sterilization: Not specified.

Use: Cultivation of paraffin bacteria, *Bacterium aliphaticum, Bacterium aliphaticum liquefaciens.*

Reference: Tausz and Peters (1919 p. 49).

1480. Simmons' Citrate Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	20.0 g.
3. NaCl	5.0 g.
4. $MgSO_4$	0.2 g.
5. $(NH_4)H_2PO_4$	1.0 g.
6. K_2HPO_4	1.0 g.
7. Sodium citrate (2.77 g. of sodium citrate, $5\frac{1}{2}$ H_2O)	2.0 g.
8. Brom-thymol blue (1.5% alcoholic)	10.0 cc.

Preparation:

(1) Dissolve 3, 4, 5, 6 and 7 in 1.
(2) Add 20.0 g. clean washed agar and autoclave at 15 pounds pressure for 15 minutes.
(3) Adjust to pH = 6.8.
(4) Add 8.
(5) Tube and slant.

Sterilization: See step (2) above.

Use: Differentiation of typhoid-colon aerogenes group. The author reported that *Escherichia coli* was markedly or completely inhibited and does not change the color of the medium. *Aerobacter aerogenes* grew luxuriantly, forming large greenish blue colonies, producing a blue color in the medium.

Variants: The dye used in the medium above was a product of National Anilin and Chemical Company. A 0.4% aqueous solution of brom-thymol blue may be used in the proportions of 20.0 cc. per liter of agar. The aqueous solution is prepared by grinding 0.1 g. of dry powder in an agate mortar with 3.2 cc. of N/20 NaOH and when solution is complete dilute to 25.0 cc. with water. The brom-thymol blue distributed by the Lamotte Chemical Products Company gave satisfactory results when used in these proportions.

Reference: Simmons (1926 p. 209).

1481. Dolt's Lactose Ammonium Phosphate Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar (purified)	30.0 g.
3. Glycerol	5.0 g.
4. Ammonium phosphate	1.0 g.
5. Lactose	10.0 g.
6. Azolitmin	1.0 g.

Preparation:

(1) Purify agar by cutting it in small pieces and soaking it in distilled water 24 hours.
(2) Dissolve (1), 2, 3 and 4 in 1.
(3) Add NaOH until neutral to phenolphthalein.
(4) Dissolve 5 in (3).
(5) Dissolve 1.0 g. of Kahlbaum's C. P. azolitmin in 100.0 cc. distilled water and boil, 15 minutes. This gives a blue solution.
(6) Add 10.0 cc. of (5) to (4).

Sterilization: Method not given.

Use: Detection of acid production in water analysis. Author reported that *B. coli* produced a red colony on this medium. Bengis cultivated *B. coli* on a similar medium.

Variants: Bengis prepared a medium as follows: He employed the medium to cultivate large numbers of *B. coli*:

(1) Dissolve 30.0 g. of agar in distilled water.
(2) Add 10.0 g. glycerol and 2.0 g.

$(NH_4)_2HPO_4$ to (1) and heat for 10 minutes in a water bath.
(3) Dissolve 10.0 g. of lactose in 2.
(4) Filter.
(5) Sterilize in the autoclave.

References: Dolt (1908 p. 625), Bengis (1916 p. 392).

1482. Harrison and VanderLeck's Aesculin Ammonium Phosphate Agar

Constituents:

1.	Distilled water	1000.0 cc.
2.	Agar	15.0 g.
3.	Glycerol	5.0 g.
4.	Ammonium phosphate	1.0 g.
5.	Iron citrate	2.5 g.
6.	Aesculin	1.0 g.

Preparation:
(1) Purify agar by cutting it in small pieces and soaking it in distilled water for 24 hours.
(2) Dissolve (1), 3, 4 and 5 in 1.
(3) Adjust reaction to +0.4.

Sterilization: Method not specified.

Use: Detection of *B. coli* and *B. typhosus.* Authors reported that *B. coli* colonies were black, while typhoid colonies were not.

Reference: Harrison and VanderLeck (1909 p. 622).

1483. Bengis' Ammonium Lactate Agar

Constituents:

1.	Distilled water	1000.0 cc.
2.	Agar	25.0 g.
3.	Na_2HPO_4	2.0 g.
4.	$CaCO_3$	10.0 g.
5.	Ammonium lactate	10.0 g.

Preparation:
(1) Dissolve 2 and 3 in 1 in the autoclave.
(2) Filter solution thru glass wool.
(3) Adjust reaction to +0.3.
(4) Distribute into liter Erlenmeyer flasks in 500.0 cc. lots. The flasks containing 5.0 g. of $CaCO_3$ each.
(5) Add 5.0 g. of ammonium lactate to each flask.
(6) Shake thoroly.

Sterilization: Sterilize in autoclave at 12 pounds pressure for 15 minutes.

Use: To cultivate large amounts of *B. coli.*

Variants:
(a) The author omitted the $CaCO_3$.
(b) Scales studied the variation in morphology of *B. coli* in the medium using only 15.0 g. agar per liter.

References: Bengis (1916 p. 393), Scales (1921 p. 595).

1484. Cohn's Ammonium Tartrate Agar (Klimmer)

Constituents:

1.	Water	1000.0 cc.
2.	K_2HPO_4	5.0 g.
3.	$MgSO_4$ (Crystalline)	5.0 g.
4.	Calcium phosphate	0.5 g.
5.	Ammonium tartrate	10.0 g.
6.	Agar	15.0 to 30.0 g.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Solidify by dissolving 15.0 to 30.0 g. of agar in (1).

Sterilization: Not specified.

Use: General synthetic culture medium.

Reference: Klimmer (1923 p. 172).

1485. Nelson's Ammonium Succinate Agar

Constituents:

1.	Distilled water	1000.0 cc.
2.	K_2HPO_4	2.0 g.
3.	Ammonium succinate	2.0 g.
4.	NaCl	2.0 g.
5.	Glucose	2.0 g.
6.	Agar	20.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Reaction of pH = 7.0 to 7.2 (unadjusted).

Sterilization: Sterilize at 10 pounds pressure for 20 minutes.

Use: Cultivation of colon-typhoid group in mass culture.

Reference: Nelson (1926 p. 373).

1486. Dolt's Lactose Lactate Agar

Constituents:

1.	Distilled water	1000.0 cc.
2.	Agar (purified)	30.0 g.
3.	Ammonium lactate	5.0 g.
4.	Na_2HPO_4	1.0 g.
5.	Lactose	10.0 g.
6.	Azolitmin (1.0% solution)	10.0 cc.

Preparation:
(1) Purify agar by cutting it into small pieces and soaking in distilled water 24 hours.
(2) Dissolve (1), 2, 3 and 4 in 1.

(3) Make neutral to phenolphthalein with NaOH.
(4) Dissolve 5 in (3).
(5) Prepare 6 by dissolving 1.0 g. Kahlbaum's C. P. azolitmin in 100.0 cc. distilled water. Boil 15 minutes. The solution is blue.
(6) Add (5) to (4).

Sterilization: Method not given.

Use: Detection of acid production in water analysis. Bengis used a similar medium for the cultivation of *B. coli* in large quantities.

Variants: (a) Bengis omitted the azolitmin solution, used 2.0 g. Na_2HPO_4 instead of 1.0 g., and used 10.0 g. ammonium lactate instead of 5.0 g. He reported that the medium gave excellent results without lactose.

References: Dolt (1908 p. 626), Bengis (1916 p. 392).

1487. Harrison and VanderLeck's Aesculin Lactate Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	15.0 g.
3. Ammonium lactate	5.0 g.
4. Na_2HPO_4	1.0 g.
5. Iron citrate	2.5 g.
6. Aesculin	1.0 g.

Preparation:
(1) Purify agar by cutting it in small pieces and soaking them in water for 24 hours.
(2) Dissolve (1), 3, 4, 5 and 6 in 1.
(3) Adjust reaction to 0.4.

Sterilization: Not specified.

Use: Detection of *B. coli* and *B. typhosus*. Author reported that *B. coli* produced black colonies, typhoid organisms did not.

Reference: Harrison and VanderLeck (1909 p. 622).

1488. Fischer's Nitrate Tartrate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar (1.25%)	12.5 g.
3. Dextrose (0.1%)	1.0 g.
4. Ammonium tartrate (0.1%)	1.0 g.
5. KNO_3 (0.05%)	0.5 g.
6. Soda (crystalline) (0.15%)	1.5 g.
7. K_2HPO_4 (0.1%)	1.0 g.
8. $CaCl_2$ (0.01%)	0.1 g.
9. $MgSO_4$ (Cr.) (0.03%)	0.3 g.
10. NaCl (0.01%)	0.1 g.
11. Fe_2Cl_6 (0.001%)	0.01 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 in 1. (7, 8, 9, 10 and 11 constitute Meyers mineral solution in the percentages given.)

Sterilization: Method not given.

Use: Bacterial count in soils.

Reference: Fischer (1910 p. 457).

1489. Sackett's Glucose Nitrate Agar

Constituents:

1. Water	1000.0 cc.
2. Glucose	20.0 g.
3. Agar	20.0 g.
4. $NaNO_3$	

Preparation:
(1) Dissolve 2 and 3 in 1.
(2) Prepare a 10.0% $NaNO_3$ solution in distilled water.
(3) Add increasing amounts of (2) to (1) so that the $NaNO_3$ concentration will be 0.0, 0.01, 0.03, 0.05, 0.1, 0.3 and 0.5%.
(4) Distribute in test tubes.

Sterilization: Sterilize in the autoclave for 5 minutes at 120°C.

Use: To study pigment production of *Azotobacter chroococcum.* Author reported that $NaNO_3$ favored the production of a brown pigment, NH_4Cl, $(NH_4)_2SO_4$, asparagin and peptone did not increase pigment production.

Reference: Sackett (1913 p. 109).

1490. Conn and Breed's Carbohydrate Nitrate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. Glucose or sucrose	10.0 g.
4. KNO_3	1.0 g.
5. $CaCl_2$	0.5 g.
6. $MgSO_4$	5.0 g.
7. K_2HPO_4	5.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Study of reduction of nitrates.

Variants:
(a) The authors omitted the $MgSO_4$ and used only 0.5 g. K_2HPO_4 instead of 5.0 g.

(b) Hucker and Wall omitted the $MgSO_4$ and used 0.5 g. K_2HPO_4 instead of 5.0 g. They used the medium to determine ammonia production from inorganic sources. Add 1.0 cc. of a 10.0% phenol and 1.0 cc. of a 1.0% (available chlorine) sodium hypochlorite solution to the surface of an agar slant. A blue color forming within 30 minutes denotes ammonia production. Also 2.0 cc. of neutral formaldehyde containing a few drops of phenolphthalein may be added to the slant. Presence of acid indicates ammonia formation.

References: Conn and Breed (1919 p. 278), Hucker and Wall (1922 p. 517), (1922 p. 485), Committee S. A. B. (1922 p. 525), (1923 p. 27).

1491. Groenewege's Sucrose Nitrate Agar

Constituents:

1. Water agar	1000.0 cc.
2. Sucrose	20.0 g.
3. KNO_3	1.0 g.
4. K_2HPO_4	0.5 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1000.0 cc. of tap water agar. (Composition not given.)

Sterilization: Method not given.

Use: Cultivation of *Phytobacter lycopersicum n. sp.* (Causing tomato rot.)

Reference: Groenewege (1913 p. 30).

1492. Czapek's Sucrose Nitrate Agar (Conn)

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. $MgSO_4$	0.5 g.
4. KCl	0.5 g.
5. K_2HPO_4	1.0 g.
6. $FeSO_4$	0.01 g.
7. $NaNO_3$	2 0 g.
8. Sucrose	30.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Method not specified.

Use: Cultivation of actinomyces.

Reference: Conn (1921 p. 21).

1493. Pinoy's Dextrin Nitrate Agar

Constituents:

1. Water	600.0 cc.
2. Agar	9.0 g.
3. Dextrin	15.0 g.
4. $CaNO_3$	2.0 g.
5. KH_2PO_4	0.5 g.
6. KNO_3	0.5 g.
7. $MgSO_4$	0.5 g.

Preparation:

(1) Prepare a 3.0% agar solution (9.0 g. agar in 300.0 cc. of water).

(2) Dissolve 3, 4, 5, 6 and 7 in 300.0 cc. of water.

(3) Mix equal parts sterile (1) and sterile (2).

Sterilization: Sterilize (1) and (2) separately, method not given.

Use: Cultivation of *B. fluorescens.*

Reference: Pinoy (1907 p. 630).

1494. Giltay's Glucose Nitrate Agar (Giltner)

Constituents:

1. Distilled water	1000.0 cc.
2. KH_2PO_4	2.0 g.
3. $MgSO_4$	2.0 g.
4. KNO_3	1.0 g.
5. $CaCl_2$	0.2 g.
6. Fe_2Cl_6 soln	2 drops
7. Agar (1.5%)	15.0 g.
8. Citric acid	5.0 g.
9. Glucose	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 8 in 800.0 cc. distilled water.

(2) Dissolve 1.5% of washed agar in (1) by boiling.

(3) Add a few drops of phenolphthalein.

(4) Add sufficient 10.0% NaOH to turn the solution a faint pink.

(5) Filter thru absorbent cotton.

(6) Dissolve 10.0 g. of glucose in (3).

(7) Mix (5) and (6) thoroly and make up to 1000.0 cc.

Sterilization: Sterilize in the autoclave at 15 pounds pressure for 10 minutes.

Use: To study denitrification.

Variants: Heinemann used 20.0 g. agar instead of 15.0 g.

References: Giltner (1921 p. 376), Heinemann (1922 p. 40).

1495. Conn and Breed's Double Sugar Nitrate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.

3. Glucose	10.0 g.
4. Lactose	5.0 g.
5. KNO_3	1.0 g.
6. $CaCl_2$	0.5 g.
7. K_2HPO_4	0.5 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Method not specified.

Use: For reduction of nitrate.

Reference: Conn and Breed (1919 p. 278).

SUBGROUP II-C. SECTION 2

Basal or complete media containing agar together with nitrogen and carbon of known chemical composition.

A_1. Basal media used with the addition of other materials.
  Miehe's Basal Asparagin Agar...... 1496
  Fred and Peterson's Basal Asparagin Agar...... 1497
  Stuart's Basal Asparagin Agar...... 1498
  Long's Basal Glycerol Asparagin Agar (Uyei)...... 1499
A_2. Complete media.
B_1. Containing amino acids or their salts.
C_1. Asparagin or sodium asparaginate employed.
D_1. No additional organic carbon added.
  Noyes' Asparaginate Agar...... 1500
  Sullivan's Salt Asparagin Agar...... 1501
  Davis' Asparagin Phosphate Agar...... 1502
  Bengis' Phosphate Asparagin Agar...... 1503
  Conn's Glucose Asparaginate Agar...... 1504
  Kisch's Glucose Asparagin Agar...... 1505
  Dolt's Lactose Asparagin Agar...... 1506
D_2. Additional organic carbon added.
E_1. Only one type of organic carbon added.
F_1. Carbohydrates added.
  Groenewege's Starch Asparagin Agar...... 1507
  Noyes' Starch Asparaginate Agar...... 1508
  Stutzer & Hartleb's Glycerol Asparagin Agar...... 1509
F_2. Alcohols added.
  Sullivan's Glycerol Asparagin Agar...... 1510
  Peklo's Mannitol Asparagin Agar...... 1511
  Thornton's Mannitol Asparagin Agar...... 1512
  Conn's Glycerol Asparaginate Agar...... 1513
F_3. Organic acids or their salts added.
  Beijerinck's Malate Asparagin Agar...... 1514
  Fraenkel's Lactate Asparagin Agar (Klimmer)...... 1515
  Voges' Lactate Asparaginate Agar...... 1516
  Giltay's Citric Acid Asparagin Agar (Löhnis)...... 1517
E_2. More than one type of organic carbon added.
  Conn's Asparaginate Agar...... 1518
  Smith's Levulose Asparaginate Agar...... 1519
  Higgins' Basal Lactose Asparagin Agar...... 1520
  Sullivan's Glycerol-Nitrate Asparagin Agar...... 1521
  Maassen's Malic Acid Asparagin Agar (Klimmer)...... 1522
  Uschinsky's Glycerol Asparaginate Agar (Klimmer)...... 1523
C_2. Other amino acids employed.
  Berthelot's Nitrate Tyrosein Agar...... 1524
  Jones' Histidin-hydro-chloride Agar...... 1525
B_2. Not containing amino acids, or their salts.
  Söhngen's Malate Urea Agar (Percival)...... 1526
  Perotti's Glucose Dicyandiamide Agar...... 1527
  Stapp's Uric Acid Agar...... 1528
  Stapp's Hippurate Agar...... 1529

1496. Miehe's Basal Asparagin Agar

Same as medium 379, but solidified by the addition of agar.

1497. Fred and Peterson's Basal Asparagin Agar

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2SO_4$	3.0 g.
3. Asparagin	1.5 g.
4. K_2HPO_4	2.0 g.
5. $CaCl_2$	0.25 g.
6. $MgSO_4$	0.25 g.
7. Agar	15.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5, 6, and 7 in 1.
(2) Dissolve one of the sterile added nutrients to sterile (1).
(3) Reaction employed varied from pH-6.5 to 7.2.

Sterilization: Method not given.

Use: To cultivate yeast found in sauerkraut.

Added nutrients: Authors added 2.0% glucose, maltose or xylose to the basic agar medium.

Variants: Authors added 2.0 or 4.0% NaCl to the basic agar solution.

Reference: Fred and Peterson (1922 p. 261).

1498. Stuart's Basal Asparagin Agar

Constituents:

1. Agar solution	1000.0 cc.
2. Asparagin	10.0 g.

Preparation:

(1) Prepare an agar solution using washed agar prepared according to medium 1398.

(2) Dissolve one of the added nutrients in (1).

(3) Adjust the reaction between pH-7.0 and 7.2.

Sterilization: Method not given.

Use: To study variation in *Bacterium typhosum.*

Added nutrients: The author added one of the following:

$NaNO_3$	2.0 g.
KNO_3	2.0 g.
NH_4NO_3	2.0 g.
sodium phosphate	2.0 g.
potassium phosphate	2.0 g.

Variants:

(a) The author used 4.0 g. asparagin and added 2.0 g. sodium phosphate, 6.0 g. ammonium lactate and 5.0 g. NaCl.

(b) The author used 3.4 g. asparagin in the basic agar solution and added 1.0 g. potassium phosphate, 10.0 g. ammonium lactate 5.0 g. NaCl, 0.2 g. $MgSO_4$, 0.1 g. $CaCl_2$ and 40.0 g. glycerol.

Reference: Stuart (1924 p. 586).

1499. Long's Basal Glycerol Asparagin Agar (Uyei)

Constituents:

1. Water	1000.0 cc.
2. Agar	25.0 g.
3. Glycerol	50.0 g.
4. Ferrous ammonium citrate	0.05 g.
5. $MgSO_4$	1.0 g.
6. NaCl	2.0 g.
7. Na_2CO_3	3.0 g.
8. KH_2PO_4	3.0 g.
9. Ammonium citrate	5.0 g.
10. Asparagin	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8, 9 and 10 in 1.

(2) Add one of the added nutrients.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli. The author reported that extracts of tubercle bacilli failed to show the presence of any growth stimulating substances. Cod liver oil concentrate exerted an indifferent action, while concentrations of 1:1000 Harris yeast vitamin, 1:100 tomato juice and orange juice, or 1:20 cabbage, exerted an acceleration of growth.

Added nutrients: The author added one of the following in various concentrations:

(a) Cod liver oil concentrate (Oscodal of Funk and Dubin).

(b) Harris yeast vitamin.

(c) Tomato juice.

(d) Orange juice.

(e) Cabbage.

(f) Tubercle bacilli extracts.

Variants: The author used the basic medium without any additions.

Reference: Uyei (1927 p. 427), (1927 p. 437).

1500. Noyes' Asparaginate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar (best)	15.0 g.
3. Sodium asparaginate	1.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

Sterilization: Method not given.

Use: General culture medium. Primarily used for study of soil organisms.

Reference: Noyes (1916 pp. 93, 94).

1501. Sullivan's Salt Asparagin Agar

Constituents:

1. Water	1000.0 cc.
2. Asparagin	10.0 g.
3. NaCl	10.0 g.
4. $MgSO_4$	3.0 g.
5. Agar	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Method not given.

Use: General synthetic culture medium. Also used to study pigment production. Author reported that most of the chromogenic bacteria did not produce color on this medium. *B. pyocyaneus* produced abundant pigment, however.

Reference: Sullivan (1905–06 p. 117).

1502. Davis' Asparagin Phosphate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. Asparagin	20.0 g.
4. $MgSO_4$	10.0 g.
5. K_2HPO_4	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Method not given.

Use: To produce chlamydospores by *Sporothrix schenckii*. Author reported that the growth of the organism was slightly accelerated if pure or impure sugars be added.

Reference: Davis (1914 p. 485), (1915 p. 179).

1503. Bengis' Phosphate Asparagin Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Asparagin	10.0 g.
3. Agar (powdered)	30.0 g.
4. Na_2HPO_4	2.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Filter into incubating flasks.

Sterilization: Sterilize in Bramhall-Dean autoclave.

Use: To cultivate large quantities of *B. coli*.

Variants:

(a) The author used 2.0 g. $(NH_4)_2HPO_4$ instead of Na_2HPO_4.

(b) Tanner described a similar medium as Dolt's asparagin agar. One of these media was composed of 100.0 cc. of distilled water, 350.0 cc. of a 3.0% purified agar solution in water, 2.5 g. asparagin and 0.5 g. Na_2HPO_4. The other medium contained 250.0 cc. distilled water, 250.0 cc. of a 3.0% purified agar solution, 6.25 g. asparagin and 1.25 g. Na_2HPO_4. Tanner reported that these media would give a good growth of *B. coli* in 24 hours.

References: Bengis (1916 p. 392), Tanner (1919 pp. 50, 65).

1504. Conn's Glucose Asparaginate Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	12.0 g.
3. Sodium asparaginate	1.0 g.
4. Glucose	1.0 g.
5. $MgSO_4$	0.2 g.
6. $NH_4H_2PO_4$	1.5 g.
7. $CaCl_2$	0.1 g.
8. KCl	0.1 g.
9. $FeCl_3$	trace.

Preparation:

(1) Dissolve 2, 5, 6, 7, 8 and 9 in 1.

(2) Adjust to 0.8 or 1.0% normal acid to phenolphthalein.

(3) May be clarified with egg or by simply heating for 30 minutes at 15 pounds pressure in such a way as not to disturb the sediment and then decanting thru cotton filter.

(4) Add 1.0 g. glucose and 1.0 g. sodium asparaginate to (3).

Sterilization: Method not given.

Use: Bacterial counts in soils. Author reported that there may be other satisfactory combinations of the salts than the one given. The amount of glucose and asparaginate were increased without varying the count.

References: Conn (1916 p. 722), Giltner (1921 p. 372).

1505. Kisch's Glucose Asparagin Agar

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $MgSO_4$	0.5 g.
4. NaCl	0.02 g.
5. $FeSO_4$	trace
6. Calcium phosphate	trace
7. Dextrose	10.0 g.
8. Asparagin	1.9 g.
9. Agar (2.0%)	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Add Na_2CO_3 until the reaction is slightly alkaline to litmus.

(3) Dissolve 9 in (2).

(4) Dissolve 8 in (3).

Sterilization: Not specified.

Use: To study nitrogen requirements for colon typhoid group.

Reference: Kisch (1918–19 p. 32).

1506. Dolt's Lactose Asparagin Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar, purified	30.0 g.
3. Asparagin	10.0 g.
4. Na_2HPO_4	2.0 g.
5. Lactose	10.0 g.
6. Azolitmin (1.0% solution)	10.0 cc.

Preparation:

(1) Purify agar by cutting it in small pieces and soaking it in distilled water for 24 hours.
(2) Dissolve (1), 2, 3 and 4 in 1.
(3) Make neutral to phenolphthalein with NaOH.
(4) Add 5.
(5) Prepare 6 by dissolving 1.0 g. of Kahlbaum's C.P. azolitmin in 100.0 g. distilled water and boil for 15 minutes. It is a blue solution after boiling.
(6) Add (5) to (4).

Sterilization: Method not given.

Use: Detection of acid forming bacteria in water analysis. Author reported that *B. coli* developed red colonies.

Reference: Dolt (1908 p. 621).

1507. Groenewege's Starch Asparagin Agar

Constituents:

1. Water agar	1000.0 cc.
2. Starch	1.0 g.
3. Asparagin	1.0 g.
4. K_2HPO_4	0.5 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1000.0 cc. tap water agar. (Composition not given.)

Sterilization: Method not given.

Use: Diastase production by *Phytobacter lycopersicum n. sp.* (causing tomato rot). Author reported that when diastase was produced, the starch disappeared.

Reference: Groenewege (1913 p. 28).

1508. Noyes' Starch Asparaginate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar (best)	15.0 g.
3. Starch	2.0 g.
4. Sodium asparaginate	1.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

Sterilization: Method not specified.

Use: General culture medium, primarily used to study organisms of the soil.

Reference: Noyes (1916 pp. 93–94).

1509. Stutzer and Hartleb's Glycerol Asparagin Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	20.0 g.
3. Asparagin	1.0 g.
4. Glycerol	1.0 g.
5. Potassium phosphate	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4, and 5 in 1.

Sterilization: Not specified.

Variants: Fraenkel used 15.0 g. agar instead of 20.0 g.

References: Stutzer and Hartleb (1897 p. 403), Fraenkel (1898 p. 10).

1510. Sullivan's Glycerol Asparagin Agar

Constituents:

1. Water	1000.0 cc.
2. Asparagin	10.0 g.
3. Glycerol	35.0 g.
4. K_2HPO_4	1.0 g.
5. $(NH_4)_3PO_4$	10.0 g.
6. $MgSO_4$	2.0 g.
7. Agar	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Medium is slightly acid due to evaporation of NH_3 in sterilization.

Sterilization: Method not given.

Use: General culture medium. Author reported that *B. typhosus* did not grow in this medium.

Reference: Sullivan (1905–06 p. 119).

1511. Peklo's Mannitol Asparagin Agar

Constituents:

1. Distilled water	500.0 cc.
2. KH_2PO_4	1.0 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4$	0.6 g.
5. NaCl	0.2 g.
6. $CaCl_2$	0.1 g.
7. Asparagin	2.0 g.
8. Mannitol	10.0 g.
9. Agar	0.75%
10. Fe_2Cl_6	trace

Preparation:

(1) Dissolve 2, 3, 4, 5, and 6 in 200.0 cc. of distilled water.

(2) To 100.0 cc. of (1) add 400.0 cc. distilled water and dissolve 2.0 g. asparagin, 1.0 g. mannitol and a trace of Fe_2Cl_6 in the mixture.

(3) From sterile (2) prepare a 0.75% agar. (Method or final sterilization not given.)

Sterilization: Sterilize (2) several times (method not given). A turbidity forms but disappears when the medium cools.

Use: Cultivation of plant actinomyces. Author reported good growth.

Reference: Peklo (1910 p. 473).

1512. Thornton's Mannitol Asparagin Agar

Constituents:

1. Water	1000.0	cc.
2. K_2HPO_4	1.0	g.
3. $MgSO_4 \cdot 7H_2O$	0.2	g.
4. $CaCl_2$	0.1	g.
5. $FeCl_3$	0.002	g.
6. KNO_3	0.5	g.
7. Asparagin	0.5	g.
8. NaCl	0.1	g.
9. Agar	15.0	g.
10. Mannitol	1.0	g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1 by steaming for 30 minutes.

(2) Neutralize to turmeric paper.

(3) Filter thru cotton-wool.

(4) Add 1.0 g. mannitol to (3).

(5) Tube in 8.0 cc. quantities.

Sterilization: Sterilize in the autoclave at 22.5 pounds pressure.

Use: Cultivation of soil forms.

References: Thornton (1922 p. 241) taken from (1923 p. 277), Cunningham (1924 p. 136).

1513. Conn's Glycerol Asparaginate Agar

Constituents:

1. Water	1000.0	cc.
2. Agar	15.0	g.
3. Glycerol	10.0	cc.
4. K_2HPO_4	0.5	g.
5. Sodium asparaginate	1.0	g.

Preparation:

(1) Dissolve 2, 3, 4, and 5 in 1.

Sterilization: Method not given.

Use: Cultivation of actinomycetes.

Reference: Conn (1921 p. 7).

1514. Beijerinck's Malate Asparagin Agar

Constituents:

1. Water.
2. Agar.
3. Sodium malate.
4. Asparagin.
5. Potassium phosphate.
6. Mohr salt $(NH_4)_2SO_4$

Preparation:

(1) Prepare a water solution of agar that has been washed free of soluble material with distilled water.

(2) Pour distilled water over the solidified mass and renew the water often.

(3) Melt and boil.

(4) Add a small amount of nutrient solution consisting of sodium malate, asparagin and potassium phosphate (amounts not specified).

(5) Boil again to remove all the air.

(6) Add a drop of neutral solution of Mohr salt, $(NH_4)_2SO_4$, containing a trace of Na_2CO_3, there should be no turbidity.

Sterilization: Not specified.

Use: Cultivation of *Spirillum desulfuricans*, *Spirillum tenue* and other sulphate reducing organisms.

Reference: Beijerinck (1895 p. 108).

1515. Fraenkel's Lactate Asparagin Agar (Klimmer)

Constituents:

1. Water	1000.0	cc.
2. K_2HPO_4	2.0	g.
3. NaCl	5.0	g.
4. Ammonium lactate	6.0	g.
5. Asparagin	4.0	g.
6. Agar	15.0 to 30.0	g.

Preparation:

(1) Dissolve 2, 3, 4, 5, and 6 in 1.

(2) Make distinctly alkaline (indicator not specified).

Sterilization: Not specified.

Use: Synthetic culture medium.

Reference: Klimmer (1923 p. 172).

1516. Voges' Lactate Asparaginate Agar

Same as medium 4416, but containing 10.0 g. of agar per liter of medium.

1517. Giltay's Citric Acid Asparagin Agar (Löhnis)

Constituents:

1. Distilled water	1000.0 cc.
2. KNO_3	2.0 g.
3. Asparagin	1.0 g.
4. Citric acid	5.0 g.
5. KH_2PO_4	2.0 g.
6. $MgSO_4$	2.0 g.
7. $CaCl_2$	0.2 g.
8. Fe_2Cl_6	trace
9. Agar (1.5%)	15.0 g.

Preparation:

(1) Dissolve 2 and 3 in 250.0 cc. of 1.
(2) Dissolve 4, 5, 6, 7 and 8 in 500.0 cc. of 1.
(3) Neutralize (2) by the addition of KOH.
(4) Mix (1) and (3) and make up to a liter.
(5) Dissolve 1.5% agar in (4).
(6) Tube.

Sterilization: Method not given.

Use: To study denitrification.

Reference: Löhnis (1913 p. 98).

1518. Conn's Asparaginate Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	12.0 g.
3. Glucose	1.0 g.
4. Sodium asparaginate	1.0 g.
5. Glycerol	10.0 g.
6. $NH_4H_2PO_4$	1.5 g.
7. $CaCl_2$	0.1 g.
8. $MgSO_4$	0.2 g.
9. KCl	0.1 g.
10. $FeCl_3$	trace

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8, 9 and 10 in 1.
(2) Reaction adjusted to 1.0% N acid to phenolphthalein.

Sterilization: Method not given.

Use: Cultivation of soil microörganisms.

Variants: Giltner used 15.0 g. agar and specified the use of fused $CaCl_2$ and $MgSO_4 \cdot 7H_2O$.

References: Conn (1917 p. 42), Giltner (1921 p. 373), Harvey (1921–22 p. 102).

1519. Smith's Levulose Asparaginate Agar

Constituents:

1. Water	1000.0 cc.
2. Levulose	20.0 g.
3. Glycerol	10.0 g.
4. Asparagin	1.0 g.
5. Tannin (preferably from Sumack)	1.0 g.
6. Potassium citrate	1.0 g.
7. Agar	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Method not given.

Use: Cultivation of *B. Acaciae.* Author reported that slime was produced.

Reference: Smith (1905 p. 383).

1520. Higgins' Basic Lactose Asparagin Agar

Solidify Basal Medium 386 by the addition of agar.

1521. Sullivan's Glycerol Nitrate Asparagin Agar

Constituents:

1. Water	1000.0 cc.
2. Asparagin	10.0 g.
3. $MgSO_4$	0.2 g.
4. K_2HPO_4	1.0 g.
5. Ammonium lactate	0.5 g.
6. NaCl	5.0 g.
7. KNO_3	0.2 g.
8. Glycerol	10.0 g.
9. Agar	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

Sterilization: Not specified.

Use: General Synthetic Medium.

Reference: Sullivan (1905–06 p. 120).

1522. Maassen's Malic Acid Asparagin Agar (Klimmer)

1. Water	1000.0	cc.
2. $MgSO_4$ (crystalline)	0.4	g.
3. Asparagin	10.1	g.
4. $CaCl_2$	0.01	g.
5. Na_2HPO_4	2.0	g.
6. Na_2CO_3 (crystalline)	2.5	g.
7. Glucose	5.0	to 10.0 g.
8. Malic acid	7.0	g.
9. Agar	15.0	to 30.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

Sterilization: Not specified.

Use: Synthetic culture medium.

Reference: Klimmer (1923 p. 172).

1523. Uschinsky's Glycerol Asparaginate Agar (Klimmer)

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	2.0 to 2.5 g.
3. $MgSO_4$ (Crystalline)	0.2 to 0.4 g.
4. NaCl	5.0 to 7.0 g.
5. Ammonium lactate	6.0 to 7.0 g.
6. $CaCl_2$	0.1 g.
7. Sodium asparaginate	3.5 g.
8. Glycerol	30.0 to 40.0 g.
9. Agar	15.0 to 30.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

Sterilization: Not specified.

Use: Synthetic culture medium.

Reference: Klimmer (1923 p. 172).

1524. Berthelot's Nitrate Tyrosine Agar

Same as medium 402, but solidified by the addition of agar.

1525. Jones' Histidin-hydro-chloride Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Histidin-hydro-chloride	1.0 g.
3. KNO_3	0.25 g.
4. $CaCl_2$	0.02 g.
5. K_2SO_4	0.2 g.
6. $MgSO_4$	0.2 g.
7. K_2HPO_4	5.0 g.
8. Agar	8.0 g.
9. Na_2SO_3 (10.0% solution)	12.0 cc.
10. Basic fuchsin solution	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 500.0 cc. of distilled water.

(2) Dissolve 6 in 100.0 cc. distilled water. Add to (1).

(3) Dissolve 7 in distilled water.

(4) Mix (2) and (3) and make up to 1000.0 cc.

(5) Wash 8.0 g. agar and add to (4).

(6) Sterilize at 15 pounds pressure for 15 minutes.

(7) Add 12.0 cc. of a 10.0% Na_2SO_3 solution.

(8) Titrate to neutral to litmus using N/10 HCl.

(9) Add 30 drops of saturated alcoholic solution of basic fuchsin.

(10) Mix thoroly and pour into plates.

Sterilization: Sterilization given in step (6) of preparation.

Use: Isolation of *B. aminophilu.* Author reported that *B. aminophilus* developed a colony 2.3 mm. in diameter, after 24 hours at 37°C., having the appearance of a clear colorless drop of water.

Reference: Jones (1918 p. 127).

1526. Söhngen's Malate Urea Agar (Percival)

Constituents:

1. Water (tap)	1000.0 cc.
2. Calcium malate	5.0 g.
3. K_2HPO_4	0.5 g.
4. Urea	10.0 g.
5. Agar	15.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of urea organisms from soil and urine. Author reported that urea splitting organisms were surrounded with small white crystals of calcium carbonate.

Reference: Percival (1920 p. 225).

1527. Perotti's Glucose Dicyandiamide Agar

Same as medium 483 but solidified by the addition of 1.5% agar.

1528. Stapp's Uric Acid Agar

Constituents:

1. Water	1000.0 cc.
2. Uric acid	0.5 g.
3. Na_2HPO_4	3.0 g.
4. KH_2PO_4	1.0 g.
5. $CaCl_2$	0.1 g.
6. $MgSO_4 \cdot 7H_2O$	0.3 g.
7. NaCl	0.1 g.
8. Fe_2Cl_6	0.01 g.
9. Agar (2.0%)	20.0 g.

Preparation:

(1) Dissolve 4, 5, 6, 7 and 8 in 100.0 cc. of water. (Meyers solution).

(2) Dissolve 2 and 3 in 450.0 cc. of water.

(3) Mix 50.0 cc. of (1) and (2) and solidify by the addition of 2.0% agar.

(4) Distribute in 50.0 cc. lots in 200.0 cc. Erlenmeyer flasks.

Sterilization: Method not given.

Use: Isolation of uric acid splitting bacteria from feces and soil, *Bac. cobayae, Bac. capri, Bac. guano, Bac. musculi, Bac. hollandicus.*

Reference: Stapp (1920 p. 3).

1529. Stapp's Hippurate Agar

Same as medium 507, but solidified by the addition of 2.0% agar.

SUBGROUP II-C. SECTION 3

Basal or complete media containing agar and peptone (or other commercial digest); additional constituents, if any, of known chemical composition.

A_1. Not containing additional materials.
  Jacobi's Peptone Agar.............. 1530
  Molisch's Manganese Peptone Agar.. 1531
  Roux and Rochaix' Peptone Agar.. 1532
  Hesse and Niedner's Nährstoff Heyden Agar.......................... 1533
  Lichtenstein's Yeast Extract Agar.. 1534
A_2. Containing additional materials.
B_1. All additional materials inorganic.
  Banning's Basal Salt Peptone Agar. 1535
  Matzuschita's Basal Sodium Chloride Peptone Agar................ 1536
  Glaessner's Nährstoff Heyden Agar. 1537
  Crendiropoulo and Panayotatou's Alkaline Peptone Agar........... 1538
  Stutzer and Hartleb's Phosphate Peptone Agar..................... 1539
  Molisch's Salt Peptone Agar........ 1540
B_2. One or more of the added materials organic.
C_1.* Not containing nitrogen in addition to the peptone.
D_1.* Only one type of additional organic carbon supplied.
E_1. Carbohydrates only added.
F_1. One carbohydrate used.
G_1. Monosaccharides only added.
H_1. Inorganic salts not added.
  Bacto Sabouraud's Dextrose Agar (Dehydrated).................... 1541
  Sabouraud's Glucose Peptone Agar (Anderson)...................... 1542
H_2. Inorganic salts added.
I_1. Containing salts of monovalent cations only.
  Matzuschita's Glucose Peptone Agar............................ 1543
  Glaessner's Glucose Peptone Agar.. 1544
  Hucker and Wall's Glucose Peptone Agar............................ 1545
  Hesse and Niedner's Glucose Peptone Agar....................... 1546
I_2. Containing salts of trivalent cations.
  Levine's Boric Acid Peptone Agar.. 1547
I_3. Containing salts of divalent cations.
  Bacto Lead Acetate Agar (Dehydrated).......................... 1548
  Lipman and Brown's Glucose Peptone Agar........................ 1549
  Waterman's Glucose Peptone Agar. 1550
  Pfeiler and Lentz' Ringer Solution Agar............................ 1551
  Harden's Glucose Peptone Agar.... 1552
G_2.* Disaccharides only added.
H_1. Sucrose employed.
  Greig-Smith's Sucrose Peptone Agar............................ 1553
  Owen's Raw Sugar Peptone Agar... 1554
  Vierling's Sucrose Peptone Agar.... 1555
H_2. Lactose employed.
  Levine's Eosine Methylene Blue Agar (Dehydrated)............... 1556
  Bacto Endo Agar (Dehydrated) Formula of Levine................ 1557
  Levine's Eosine Methylene Blue Agar............................ 1558
  Levine's Endo Agar................ 1559
  Robin's Lactose Peptone Agar...... 1560
  Harvey's Lactose Peptone Agar.... 1561
H_3. Maltose employed.
  Sabouraud's Maltose Peptone Agar (Wurtz).......................... 1562
G_3. Polysaccharides, only, added.
  Molisch's Dextrin Peptone Agar.... 1563
  Noyes' Starch Peptone Agar........ 1564
  Vierling's Starch Peptone Agar..... 1565
  Gibson's Starch Peptone Agar (Harvey)........................ 1566
F_2. Two or more carbohydrates used.
  Bacto Eosine Methylene Blue Agar (Dehydrated).................... 1567
  Levine's Rosolic Acid China Blue Peptone Agar................... 1568
E_2.* Alcohols only added.
  Sabouraud's Basal Glycerol Peptone Agar (Park, Williams & Krumwiede)......................... 1569
  Omelianski's Alcohol Peptone Agar. 1570
  Robinson and Rettger's Glycerol Opsine Agar..................... 1571
  Hesse's Glycerol Nährstoff Heyden Agar............................ 1572
  Molisch's Glycerol Peptone Agar... 1573
  Spengler's Glycerol Somatose Agar. 1574
E_3. Organic acids or their salts added.
  Omelianski's Formate Peptone Agar. 1575

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* See page 444 for C_2 and D_2.

* See G_3 and E_3.

Boekhout and de Vries Tartrate Peptone Agar 1576
Dawson's Butter Soap Peptone Agar 1577
Harvey's Phenol Peptone Agar 1578

D_2. More than one type of additional organic carbon supplied.

E_1. Carbohydrates and alcohols added.
Bacto Malt Extract Agar (Synthetic) (Dehydrated) 1579
Harvey's Glucose Glycerol Agar 1580

E_2. Carbohydrates and organic acids added.
Beijerinck's Glucose Peptone Agar. 1581
Sarbouraud's Glucose Peptone Agar (Serena) 1582
Chantemesse's Phenol Peptone Agar (Bezançon) 1583
Boekhout and de Vries' Maltose Peptone Agar 1584
Cheyney's Maltose Peptone Agar 1585
Sabouraud's Glycerol Peptone Agar 1586

E_3. Other combinations of organic carbon added.
Robinson and Rettger's Glycerol Opsine Citrate Agar 1587
Botelho's Lacto-phenol Peptone Agar 1588

C_2. Containing nitrogen in addition to peptone.

D_1. Inorganic nitrogen added.
Sullivan's Ammonium Lactate Peptone Agar 1589
Lipman and Brown's Nitrate Peptone Agar 1590
Beijerinck's Ferric Ammonium Citrate Peptone Agar (Janke) 1591
Vierling's Nitrate Peptone Agar 1592
Heinemann's Asparagin Peptone Agar 1593

D_2. Inorganic nitrogen not added; additional organic nitrogen supplied.
MacConkey's Basal Bile Salt Peptone Agar 1594
Harrison and van der Leck's Aesculin Bile Salt Agar 1595
Harvey's Brilliant Green Bile Salt Agar 1596

1530. Jacobi's Peptone Agar

Constituents:

1. Water	1500.0 cc.
2. Meat peptone (Kemmerich's)	7.5 g.
3. Peptone (siccum)	15.0 g.
4. Agar-agar	15.0 to 22.5 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1 by heating over a free flame.
(2) Make up to the original volume by the addition of water.
(3) Add Na_2CO_3 or sodium phosphate until the reaction is slightly alkaline.
(4) Pour into a flask and steam until the albuminous material still to be removed is completely separated. (Usually 2 hours if sodium phosphate is used and longer if Na_2CO_3.)
(5) Filter thru cotton, using compressed air to effect a fast filtration.
(6) Distribute into smaller flasks.
(7) Distribute sterile (6) into sterile test tubes that have been autoclaved in the autoclave for 2½ hours. (The tubes are contained in an enamel container in the autoclave. The inner temperature reaching about 150°C.)

Sterilization: Heat (6) in streaming vapor for two hours (if the glassware used has been sterilized in the autoclave).

Use: General culture medium.

Reference: Jacobi (1888 p. 538).

1531. Molisch's Manganese Peptone Agar

Constituents:

1. Water (Moldau River)	1000.0 cc.
2. Manganese peptone	0.5 g.
3. Agar	10.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Pour in plates.

Sterilization: Method not given.

Use: Cultivation of iron bacteria, *Leptothrix ochracea*.

Reference: Molisch (1910 p. 36).

1532. Roux and Rochaix' Peptone Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	25.0 g.
3. Peptone	10.0 to 15.0 g.

Preparation:

(1) Soak 25.0 g. of chopped agar for 24 hours in 500.0 cc. of water acidulated by the addition of 6.0% HCl. Stir occasionally.
(2) Wash thoroly with water.

(3) Soak the agar for 24 hours in 500.0 cc. of a 5.0% ammonia solution.
(4) Wash thoroly.
(5) Place in a liter of distilled water and heat until the agar is dissolved.
(6) Add 50.0 cc. of water containing 10.0 to 15.0 g. peptone.
(7) Neutralize by the addition of a saturated solution of $NaHCO_3$.
(8) Pass thru flannel and then filter using a hot water funnel.
(9) Distribute into flasks or tubes.

Sterilization: Sterilize at 115° to 120° for 30 minutes.

Use: General culture medium.

Variants:

(a) Noyes dissolved 15.0 g. of the best agar and 0.05 g. of Witte's peptone in 1000.0 cc. of water.
(b) Harvey dissolved 18.0 g. of agar and 30.0 g. peptone in 1000.0 cc. of water. This medium was used to cultivate hyphomycetes.
(c) Mortensen solidified medium 560 by the addition of 2.0 or 2.5% agar.
(d) Moll used a 2.0% Witte peptone solution solidified with 2.0% agar as a basal medium and added a variety of inorganic salts to study the effect of salts on the growth of molds. He reported that the toxicity of salts is an additive characteristic of the ion. The toxicity depends on the solubility and the ionization of the salt.

References: Roux and Rochaix (1911 p. 115), Noyes (1916 pp. 93–94), Harvey (1921–22 p. 102), Mortensen (1909 p. 523), Moll (1920 p. 258).

1533. Hesse and Niedner's Nährstoff-Heyden Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	12.5 g.
3. Nährstoff-Heyden	7.5 g.

Preparation:

(1) Mix 3 with a little cold distilled water forming a paste.
(2) Dissolve 2 and (1) in 1.
(3) Filter thru cotton or paper in the steamer or hot water funnel.
(4) Adjustment of reaction not required.
(5) Pour in plates.

Sterilization: Not specified.

Use: The authors used the medium primarily for water analysis, but various investigators have used the same or similar media for a large variety of purposes.

Variants:

(a) Wimmer used the medium with only 980.0 cc. water instead of 1000.0 cc. to study nitrification.
(b) Klimmer used 8.0 g. Nährstoff-Heyden and 13.0 g. agar.

References: Hesse and Niedner (1898 pp. 454–462), Wimmer (1904 p. 139), Smith (1905 p. 196), Tanner (1919 p. 50), Percival (1920 p. 121), Klimmer (1923 p. 194).

1534. Lichtenstein's Yeast Extract Agar

Same as medium 518, but solidified by the addition of agar.

1535. Banning's Basal Salt Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. KH_2PO_4	3.0 g.
3. $MgSO_4$	2.0 g.
4. Peptone	10.0 g.
5. Agar	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Do not adjust the reaction.
(3) Dissolve one of the added nutrients in (1).

Sterilization: Heat to 75°C. to sterilize (method not given).

Use: To study oxalic acid formation by *Bact. xylinum*, *Bact. aceti oxalici* and *Bact. diabeticum*. Author reported that when nitrogenous materials were added oxalic acid was not produced.

Added nutrients: The author added one of the following materials:

methyl alcohol	10.0 g.
ethyl alcohol	30.0 g.
propyl alcohol	20.0 g.
butyl alcohol	10.0 g.
amyl alcohol	5.0 g.
ethylene glycocol	10.0 g.
glycerol	10.0 g.
erythritol	10.0 g.
mannitol	10.0 g.
sodium salt of acetic acid	10.0 g.
propionic acid	10.0 g.
butyric acid	5.0 g.

isobutyric acid	5.0 g.
valearic acid	5.0 g.
glyoxalic acid	2.5 g.
lactic acid	10.0 g.
malonic acid	2.5 g.
benzoic acid (potassium salt)	5.0 g.
salicilic acid	5.0 g.
pyrotaric acid	2.5 g.
malic acid	10.0 g.
tartaric acid	10.0 g.
salicylic acid	5.0 g.
glycocol (sodium salt)	10.0 g.
leucine (sodium salt)	10.0 g.
hippuric acid	5.0 g.
sarcosine	2.5 g.
uric acid (potassium salt)	5.0 g.
Urea	10.0 g.
creatin	2.5 g.
creatinin	2.5 g.
glucose	20.0 g.
levulose	20.0 g.
galactose	20.0 g.
maltose	20.0 g.
sucrose	20.0 g.
lactose	20.0 g.
raffinose	20.0 g.
rhamnose	10.0 g.
arabinose	20.0 g.
starch (wheat)	10.0 g.
inulin	10.0 g.
glycogen	10.0 g.
dextrin	10.0 g.
gum arabic	10.0 g.

Reference: Banning (1902 p. 395–427).

1536. Matzuschita's Basal Sodium Chloride Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Agar (2.0%)	20.0 g.
3. Peptone (1.0%)	10.0 g.
4. NaCl (0.5%)	5.0 g.

Preparation:

(1) Prepare a watery infusion of one of the materials given under added nutrients.

(2) Add 2.0% agar, 1.0% peptone and 0.5% NaCl to (1).

(3) Neutralize (indicator not given).

Sterilization: Not specified.

Use: Cultivation of intestinal bacteria. Similar media were used for the cultivation of various other organisms.

Added nutrients: The author prepared infusion from one of the following materials:

intestinal mucosa
liver
pancreas
spleen
feces (human)
brain
rice
pea

Variants:

(a) The author used undiluted bile, urine or beer wort instead of infusion listed above.

(b) Matzuschita cultivated spore forming bacilli, *Clostridium butyricum*, *Bacillus oedematis maligni*, *Bacillus anthracis symptomatici*, *Bacillus sporogenes*, *Bacillus botulinus*, on a medium prepared as follows:

(1) Dissolve 10.0 g. Koch's meat peptone, 20.0 g. agar, 5.0 g. NaCl, and 2.0% glucose (the glucose may be omitted) in 1000.0 cc. water by boiling in a steamer.

(2) Neutralize.

(3) Filter.

(4) Sterilize in the steamer on from 2 to 5 successive days for 15 to 30 minutes.

(5) Incubate for 2 days at 37°C. to test sterility.

(c) Sullivan used the basal solution with 20.0 g. peptone instead of 10.0 g., as a general culture medium for *Microspira comma*, *B. anthracis*, *B. pneumoniae*, photogenic and chromogenic bacteria. He reported slow growth and slight pigment production.

(d) Harvey used the basic solution with 10.0 to 20.0 g. peptone added. He merely stated that the solution be solidified by the addition of agar, not giving the amount employed.

(e) Teague and Deibert reported that a medium containing peptone, NaCl and agar would not support the growth of Unna-Ducrey bacillus.

References: Matzuschita (1901–02 p. 214), (1902 p. 288), Sullivan (1905–06 p. 114), Harvey (1921–22 p. 101), Teague and Deibert (1922 p. 70).

1537. Glaessner's Nährstoff-Heyden Agar

Constituents:

1. Water	1000.0 cc.
2. Agar (pulverized)	15.0 g.

3. Nährstoff-Heyden	10.0 g.
4. NaCl	5.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

Sterilization: Method not given.

Use: Cultivation of diphtheria bacilli. Author reported better growth on Loeffler's blood serum.

Reference: Glaessner (1900 p. 729).

1538. Crendiropoulo and Panayotatou's Alkaline Peptone Agar

Constituents:

1. Water		190.0 cc.
2. Peptone		5.0 g.
3. NaOH (10.0%)		10.0 cc.
4. Agar medium	agar	3.0 g.
	peptone	1.0 g.
	NaCl	0.5 g.
	H_2O	100.0 cc.

Preparation:

(1) Dissolve 5.0 g. of peptone (Witte or Chepoteau) in 190.0 cc. of water.

(2) Add 10.0 cc. of 10.0% solution of NaOH (8.0 cc. if using Witte's peptone) and heat 3 to 5 minutes.

(3) Cool and filter thru paper.

(4) Prepare neutral peptone agar by dissolving 3.0 g. agar, 1.0 g. peptone and 0.5 g. NaCl in 100.0 cc. water.

(5) Mix 4 parts sterile (4) with 6 parts sterile (5) and pour in sterile plates.

Sterilization: Sterilize (4) at 100°C. for 30 minutes. Method of sterilization of (5) not given.

Use: Diagnosis of cholera. Author reported that vibrio colonies are round, semi-transparent, bluish at first and later whitish.

Reference: Crendiropoula and Panayotatou (1910 p. 249).

1539. Stutzer and Hartleb's Phosphate Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	20.0 g.
3. Peptone	20.0 g.
4. Potassium phosphate	1.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Neutralize by the addition of soda (indicator not specified).

(3) Add 0.5 g. Na_2CO_3 to (2).

Sterilization: Method not given.

Use: Used by Stutzer and Hartleb in an attempt to cultivate the foot and mouth disease bacterium. Temple and others used similar media to determine bacterial counts in soil.

Variants: Temple used 0.1 g. K_2HPO_4, 1.0 g. peptone and 15.0 g. of agar per liter.

References: Stutzer and Hartleb (1897 p. 404), Temple (1912 p. 206), Brown (1913 p. 499).

1540. Molisch's Salt Peptone Agar

Constituents:

1. Distilled water	1000.0 cc.
2. $MgSO_4$	0.5 g.
3. K_2HPO_4	0.5 g.
4. $FeSO_4$	trace
5. Peptone	10.0 g.
6. Agar	18.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of purple bacteria.

Reference: Molisch (1907 p. 11).

1541. Bacto Sabouraud's Dextrose Agar (Dehydrated)

Constituents:

1. Water	
2. Peptone (Bacto)	10.0 g.
3. Glucose (Bacto)	40.0 g.
4. Agar (Bacto)	15.0 g.

Preparation:

(1) Dissolve 65.0 g. of Bacto Sabouraud's Dextrose Agar (dehydrated) in 1000.0 cc. of distilled water by boiling.

(2) Distribute as desired.

(3) If sterilized for 20 minutes at 15 pounds pressure pH = 5.6±.

Sterilization: Sterilize as desired. Avoid excess heat on account of high acidity of medium.

Use: General culture medium.

Reference: Digestive Ferments Co. (1925 p. 14).

1542. Sabouraud's Glucose Peptone Agar (Anderson)

Constituents:

1. Water	1000.0 cc.
2. Agar	20.0 g.
3. Peptone	10.0 g.
4. Glucose	40.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Adjust to +2 acid with N/1 HCl.
(3) Add 4.
(4) Tube in sterile tubes.

Sterilization: Sterilize for 10 minutes at 5 pounds pressure.

Use: To isolate yeast found in human intestinal tract. Different investigators used similar media to cultivate yeast, molds and other organisms.

Variants:

(a) Bezançon used 18.0 g. agar instead of 20.0 g. The medium was used to cultivate hyphomycetes, *Sporotrichum beurmanni*.
(b) Harvey cultivated tineae, molds and sporothrix on a medium prepared as follows:
  (1) Prepare: Granulated peptone 10; commercial glucose 40; agar 18; water 1000.
  (2) Raise slowly in the autoclave to a temperature of 120°C. and then allow to fall to 100°C.
  (3) Shake, to mix, at a temperature of 100°C.
  (4) Have in readiness 2 one-liter flasks with funnels and thick, filter paper.
  (5) Place these flasks in the hot autoclave.
  (6) Filter.
  (7) Keep the unfiltered medium hot.
  (8) Replace the funnels and filter paper by new funnels with filter paper as soon as filtration becomes slow.
    NOTE: The filtration of this medium is particularly difficult and slow.
  (9) Distribute the filtrate into test tubes.
    NOTE: The tubes should be capped during incubation (which may be long) but may be placed in a covered receptacle with its cover only just open.
  (10) No adjustment of reaction of this medium required.

References: Anderson (1917 p. 343), Tanner (1919 p. 51), Bezançon (1920 p. 646), Harvey (1921–22 pp. 101, 110).

1543. Matzuschita's Glucose Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.
4. Agar	12.0 g.
5. Glucose (2.0%)	20.0 g.

Preparation:

(1) Prepare ordinary nutrient agar using water instead of bouillon. (Peptone and NaCl in amounts indicated were assumed to be the constituents of nutrient agar.)
(2) Add 2.0% glucose to (1).

Sterilization: Not specified.

Use: Cultivation of mammalian and chicken tubercle bacilli. Author reported that the mammalian types grew poorly. Chicken types gave colonies of 1.5 mm. after six days.

Variants:

(a) Truche and Cotoni solidified variant (a) medium 596 by the addition of agar.
(b) Harvey solidified medium 595 with agar.

References: Matzuschita (1899 p. 128), Truche and Cotoni (1911 p. 480), Harvey (1921–22 p. 109).

1544. Glaessner's Glucose Peptone Agar

Same as medium 1537 but contains 1.0% glucose. The author also described a medium the same as 1537, containing in addition 10.0 g. peptone and 10.0 g. glucose.

1545. Hucker and Wall's Glucose Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone	40.0 g.
3. Glucose	2.0 g.
4. K_2HPO_4	5.0 g.
5. Agar	15.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Tube.

Sterilization: Method not given.

Use: To determine ammonia production by organisms. Add 1.0 cc. of a 10.0% phenol and 1.0 cc. of a 1.0% (available chlorine) sodium hypochlorite solution to the surface of the agar slant. A blue color developing within 30 minutes denotes ammonia production.

Reference: Hucker and Wall (1922 p. 516), (1922 p. 485).

1546. Hesse and Niedner's Glucose Peptone Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	12.5 g.
3. Peptone (Gehe & Co.)	10.0 g.
4. Dextrose	1.0 g.
5. Sodium phosphate (amphoteric)	0.5 g.

Preparation:

(1) Dissolve 2, 3, 4 and 0.5 g. amphoteric reacting sodium phosphate in 1 by boiling.

Sterilization: Not specified.

Use: Used in water analysis.

Reference: Hesse and Niedner (1898 pp. 454–462).

1547. Levine's Boric Acid Peptone Agar

Constituents:

1. Water	1000.0 g.
2. Peptone (1.0%)	10.0 g.
3. Agar (1.5%)	15.0 g.
4. K_2HPO_4 (0.3%)	3.0 g.
5. Glucose (0.05%)	0.5 g.
6. Boric acid (0.63%)	6.3 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Differentiation of *Bact. coli* and *Bact. aerogenes*. Author reported that *Bact. aerogenes* did not show growth. *Bact. coli* grew luxuriantly.

Reference: Levine (1911 p. 22).

1548. Bacto Lead Acetate Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Peptone (Bacto)	10.0 g.
3. Agar (Bacto)	12.0 g.
4. Glucose (Bacto)	1.0 g.
5. NaCl	5.0 g.
6. Lead acetate	0.1 g.

Preparation:

(1) Dissolve 20.0 g. of Bacto Lead Acetate Agar (dehydrated) in 1000.0 cc. of distilled water.

Sterilization: Sterilize in the usual manner.

Use: Primarily used for differentiating *Sal. paratyphi* from *Sal. schotmuelleri*. Used also in the study of other organisms of this group.

Reference: Digestive Ferments Co. (1925 p. 12).

1549. Lipman and Brown's Glucose Peptone Agar

Constituents:

1. Water (tap)	1000.0 cc.
2. Dextrose	10.0 g.
3. K_2HPO_4	0.5, 1.0 or 1.5 g.
4. $MgSO_4$	0.2 g.
5. Peptone	0.05 g.
6. Agar	20.0 g.

Preparation:

(1) Dissolve 2, 4, 5 and 6 in 1.

(2) To 1000.0 cc. of (1) add 0.5 g. of K_2HPO_4. This is medium A.

(3) To 1000.0 cc. of (1) add 1.0 g. K_2HPO_4. This is medium B.

(4) To 1000.0 cc. of (1) add 1.5 g. K_2HPO_4. This is medium C.

(5) To 1000.0 cc. of (1) add 0.5 g. K_2HPO_4, and 0.5 cc. of N/1 HCl. This is medium D.

(6) To 1000.0 cc. of (1) add 0.5 g. K_2HPO_4 and 1.0 cc. N/1 HCl. This is medium E.

Sterilization: Not specified.

Use: Bacterial count of soil. Author reported that Media A, B, D and E or combinations (2), (3), (5) and (6) gave practically the same results. The reaction with 1.5 g. K_2HPO_4 seemed to inhibit development.

Variants:

(a) Authors used 0.5 g. K_2HPO_4 and 0.1 to 0.5 g. peptone, instead of amounts used above.

(b) Waksman used 1.0 g. KH_2PO_4, 5.0 g. peptone and 25.0 g. agar instead of amounts used by Lipman and Brown.

References: Lipman and Brown (1910 pp. 447, 451, 592), Tanner (1919 pp. 49, 66), Heinemann (1922 pp. 38, 40), Waksman (1922 p. 340).

1550. Waterman's Glucose Peptone Agar

Constituents:

1. Agar solution	1000.0 cc.
2. Glucose (2.0%)	20.0 g.

3. Peptone (0.2%)	2.0 g.
4. KH_2PO_4 (0.1%)	1.0 g.
5. $CaCO_3$ (0.2%)	2.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1000.0 cc. of agar solution.

Sterilization: Method not given.

Use: To study acid production by acetic acid bacteria, *Acetobacter melanogenum*, *B. Pasteurianum*, *B. rancens*, *B. xylinum* and acetic acid bacteria from beer. *B. xylinum* and *Acetobacter melanogedum* produced acid. The remaining bacteria listed produced no or very little acid.

Variants: Janke gives this medium specifying that the agar be a 2.0% agar solution in water.

References: Waterman (1913 p. 453), Janke (1916 p. 6).

1551. Pfeiler and Lentz's Ringer Solution Agar

Constituents:

1. Water	1000.0 cc.
2. NaCl	10.0 g.
3. KCl	0.2 g.
4. $CaCl_2$	0.2 g.
5. Sodium bicarbonate	0.1 g.
6. Glucose	1.0 g.
7. Agar	15.0 g.
8. Peptone	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Add agar and peptone to (1) and boil three hours.

(3) Cool to 50°C. and clarify with egg albumin powder. Add albumin, boil and filter.

Sterilization: Method not given.

Use: General inexpensive culture medium. Author reported that organisms retained their characteristic growth, their pathogenicity, their ability to produce pigment, their agglutinative ability and their gram stain on this medium. (Messerschmidt in Centr. f. Bakt., 68: 107–111 1913, compares this medium with meat extract agar and finds meat extract medium far superior. This medium does not give characteristic reaction when used as a base for Endo Fuchsin agar, Conradi-Drigalski agar or Loeffler's Malachite green agar according to Messerschmidt.)

Variants: Park, Williams and Krumwiede used 1.0 g. $NaHCO_3$ with 1.0 or 2.0% peptone and 1.5 or 2.0% agar instead of amounts indicated above.

References: Pfeiler and Lentz (1913 p. 123), Park, Williams and Krumwiede (1924 p. 122).

1552. Harden's Glucose Peptone Agar

Constituents:

1. Water	500.0 cc.
2. Glucose	10.0 g.
3. Peptone (Witte)	5.0 g.
4. Chalk	5.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add sterile 4 to sterile (1).

Sterilization: Sterilize (1); method not given.

Use: To study fermentation of glucose.

Reference: Harden (1905 p. 488).

1553. Greig-Smith's Sucrose Peptone Agar

Same as medium 613 but solidified by the addition of 2.0% agar.

1554. Owen's Raw Sugar Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Agar (2.0%)	20.0 g.
3. Sucrose (second 80°) (10.0%)	100.0 g.
4. Peptone (1.0%)	10.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Bacterial count of cane sugar products.

Reference: Owen (1914 p. 338).

1555. Vierling's Sucrose Peptone Agar

Same as 622 but solidified by the addition of 2.0% agar.

1556. Levine's Eosine Methylene Blue Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Peptone, Bacto	10.0 g.
3. Agar, Bacto	15.0 g.
4. K_2HPO_4	2.0 g.
5. Lactose, Bacto	10.0 g
6. Eosine	0.4 g.
7. Methylene Blue	0.1 g.

Preparation:

(1) Dissolve 37.5 g. of Levine's Eosine Methylene Blue Agar (Dehydrated) in 1000.0 cc. of water by boiling, or better autoclaving.

(2) If sterilized at 15 pounds for 20 minutes pH = 7.1±.

Sterilization: Sterilize in the usual manner.

Use: Differentiation of the coli-aerogenes group.

Reference: Digestive Ferments Co. (1925 p. 10).

1557. Bacto Endo Agar (Dehydrated) Formula of Levine

Constituents:

1. Distilled water	
2. K_2HPO_4	3.5 g.
3. Peptone, Bacto	10.0 g.
4. Agar, Bacto	15.0 g.
5. Lactose, Bacto	10.0 g.
6. Na_2SO_3	2.5 g.
7. Fuchsin, Basic, (10.0% alc. soln.)	5.0 cc.

Preparation:

(1) Dissolve 41.5 g. of Bacto Endo Agar (Dehydrated) in 1000.0 cc. of distilled water by using as little heat as possible, autoclaving is recommended.

(2) If sterilized at 15 pounds for 20 minutes pH = 7.5 ±.

Sterilization: Sterilize in the usual manner.

Use: Differentiation of colon-typhoid group.

Reference: Digestive Ferments Co. (1925 p. 11).

1558. Levine's Eosine Methylene Blue Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Difco)	10.0 g.
3. K_2HPO_4	2.0 g.
4. Agar	15.0 g.
5. Lactose 10.0 g. or sterile 20.0% solution)	50.0 cc.
6. Eosine (yellowish 2.0% aq. solution)	20.0 cc.
7. Methylene blue ($\frac{1}{2}$% aq. solution)	20.0 cc.

Preparation:

(1) Dissolve 2, 3 and 4 in 1 by boiling.

(2) Make up loss due to evaporation.

(3) Place measured quantities in flasks.

(4) Just prior to use add to each 100.0 cc. of sterile (3) 1.0 g. of sterile lactose or 5.0 cc. of 20.0% sterile lactose solution. Also to each 100.0 cc. add 2.0 cc. of a 2.0% aqueous solution of eosin (yellowish) and 2.0 cc. of a 0.5% aqueous methylene blue solution.

(5) Mix thoroly and pour into sterile petri dishes. Allow to harden in the incubator.

Sterilization: Sterilize (3) at 15 pounds pressure for 15 minutes.

Use: To differentiate between *B. coli* and *B. aerogenes*. Author reported that *Bact. coli* colonies well isolated 3–4 mm. in diameter. Colonies flat, sometimes concave, dark and sometimes with a green metallic sheen. *Bact. aerogenes* isolated colonies 4–6 mm. in diameter; neighboring colonies run together quickly, colonies raised and convex; centers deep brown. By reflected light colonies are much lighter than *Bact. coli*.

Variants: Skinner and Murray added 1:100,000 crystal violet to the medium to inhibit the development of spreaders.

References: Levine (1918 p. 43), Tanner (1919 p. 54), Levine (1921 p. 117), Harvey (1921–22 p. 92), A. P. H. A. (1923 p. 96), (1925 p. 99), Skinner and Murray (1924 p. 590).

1559. Levine's Endo Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Difco)	10.0 g.
3. Dipotassium phosphate (K_2HPO_4)	2.0 to 5.0 g
4. Agar	15.0 to 30.0 g.
5. Lactose	10.0 g.
6. Fuchsin	(10.0% soln.)
7. Na_2SO_3	(10.0% soln.)

Preparation:

(1) Boil 2, 3 and 4 in 1 until solution is complete.

(2) Make up any loss due to evaporation.

(3) Not necessary to filter or adjust the reaction.

(4) Distribute in measured quantities and sterilize.

(5) When ready for use add 1.0 g. of sterile lactose, or 5.0 cc. of a 20.0% sterile lactose solution, 0.5 cc. of a 10.0% (saturated) alcoholic solution of

basic fuchsin, and 2.5 cc. of a freshly prepared 10.0% Na_2SO_3 solution to each 100.0 cc. of sterile (4).

(6) Pour into plates and allow to harden in the incubator.

Use: Direct isolation or confirmation of presumptive test for lactose fermenters.

Variants:

(a) Harvey specified the use of 3.5 g. K_2HPO_4 and 20.0 g. agar.

(b) Harris specified the use of 3.5 g. K_2HPO_4 and 15.0 g. agar.

References: Levine (1921 p. 115), Harvey (1921–22 p. 93), Harris (1925 p. 280).

1560. Robin's Lactose Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	32.0 g.
3. Peptone (Collas)	20.0 g.
4. Sodium phosphate	0.4 g.
5. Blue, soluble	
6. Lactose	40.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1 by heating at 115° in the autoclave for 5 to 10 minutes.

(2) Add 1.0 cc. of a 1.0% aqueous solution of soluble blue to boiling (1).

(3) Add sufficient tenth normal KOH solution to decolorize (2).

(4) Add 40.0 g. lactose to (3).

(5) Filter.

(6) Distribute in tubes.

Sterilization: Sterilize at 105°C. for 15 minutes.

Use: Differentiation of coli and typhoid bacilli. Author reported that coli colonies colored the medium blue while typhoid colonies were colorless.

Reference: Robin (1897 p. 50).

1561. Harvey's Lactose Peptone Agar

Same as medium 607 but solidified by the addition of agar.

1562. Sabouraud's Maltose Peptone Agar (Wurtz)

Constituents:

1. Water	1000.0 cc.
2. Maltose	38.0 g.
3. Peptone	5.0 to 8.0 g.
4. Agar	14.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1 as in the preparation of ordinary nutrient agar.

Sterilization: Method not given.

Use: Cultivation of molds.

Variants:

(a) Harvey used 18.0 g. agar, 40.0 g. of commercial French maltose and 10.0 g. of Chassaing peptone.

References: Wurtz (1897 p. 47), Harvey (1921–22 p. 110), Stitt (1923 p. 208).

1563. Molisch's Dextrin Peptone Agar

Constituents:

1. Water (Moldau)	1000.0 cc.
2. Agar	18.0 g.
3. Peptone	5.0 g.
4. Dextrin	5.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

Sterilization: Method not given.

Use: Cultivation of purple bacteria.

Reference: Molisch (1907 p. 11).

1564. Noyes' Starch Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Agar (best)	15.0 g.
3. Starch	2.0 g.
4. Peptone	0.05 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: General culture medium. Used primarily to study soil organisms.

Reference: Noyes (1916 pp. 93, 94).

1565. Vierling's Starch Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.1 g.
5. $FeCl_3$	trace
6. NaCl	trace
7. Starch	5.0 g.
8. Peptone	10.0 g.
9. Agar	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

(2) Solidify with agar (amount not specified).

Sterilization: Not given.

Use: To study amylase production by mycobacteria. Author reported that the agar was slightly turbid. The medium was slightly cleared around mycobacteria colonies. The clearing was more noticeable if the starch content was increased 1.5 or 2.0%. The starch was dissolved. No color was produced near the colonies when Lugol's solution was added.

Reference: Vierling (1920 p. 204).

1566. Gibson's Starch Peptone Agar (Harvey)

Constituents:

1. Water	1000.0 cc.
2. Peptone	20.0 g.
3. $NaHCO_3$	1.5 g.
4. Agar	30.0 g.
5. Potato starch	10.0 g.
6. Litmus	

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Filter, while hot, thru well-wetted thick filter paper.
(3) Make a suspension of 10.0 g. potato starch with a little of the hot filtrate and add to the bulk of the filtrate.
(4) Mix.
(5) Add sterile litmus solution to sterile (4) to give the desired color.

Sterilization: Sterilize (4) at 100°C. on each of three successive days.

Use: Detection of *V. cholerae.*

Reference: Harvey (1921–22 p. 112).

1567. Bacto Eosine Methylene Blue Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Peptone, Bacto	10.0 g.
3. Agar, Bacto	15.0 g.
4. Lactose, Bacto	5.0 g.
5. Sucrose, Bacto	5.0 g.
6. K_2HPO_4	2.0 g.
7. Eosine	0.2 g.
8. Methylene Blue	0.05 g.

Preparation:

(1) Dissolve 37.0 g. of Bacto Eosine Methylene Blue Agar (dehydrated) in 1000.0 cc. water by boiling or autoclaving.

Sterilization: Sterilize in the usual manner.

Use: Isolation of *Esch. coli, Esch. Ebert, Esch. typhi,* etc.

Reference: Digestive Ferments Co. (1925 p. 11).

1568. Levine's Rosolic Acid China Blue Peptone Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	15.0 g.
3. Peptone	10.0 g.
4. K_2HPO_4	4.0 g.
5. Lactose, 20% soln	50.0 cc.
6. Glucose, 5% soln	10.0 cc.
7. Rosolic acid (1.0% in 90.0% alcohol)	10.0 cc.
8. China-blue (0.5% in water)	10.0 cc.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) pH = 7.4 to 7.5, requires no adjustment.
(3) Need not be filtered if used on plates.
(4) Distribute in 100.0 cc. lots.
(5) To melted sterile (4) add 5.0 cc. sterile 5, 1.0 cc. of sterile 6, 1.0 cc. of 7 and 1.0 cc. of 8 per 100.0 cc.
(6) Mix thoroly and pour in sterile plates.

Sterilization: Sterilize (4), method not given.

Use: To isolate dysentery bacilli. The author reported that the dyes such as eosin, methylene blue, the fuchsin-sulphite indicator, and an excess of Rosolic acid and china blue inhibited many dysentery types.

Variants: Harvey used 5.0 g. peptone instead of 10.0 g.

Reference: Levine (1920 p. 39), Harvey (1921–22 p. 88).

1569. Sabouraud's Basal Glycerol Peptone Agar (Park, Williams & Krumwiede)

Constituents:

1. Water	1000.0 cc.
2. Peptone (1.0 to 2.0%)	10.0 to 20.0 g.
3. Glycerol (0.5%)	5.0 g.
4. Agar to solidify	

Preparation:

(1) Dissolve 2, 3 and one of the added nutrients in 1.
(2) Solidify by the addition of agar.
(3) Do not adjust the reaction.

Sterilization: Not specified.

Use: Cultivation of molds.

Added nutrients: The author added 2.0% glucose or 2.0% maltose.

Reference: Park, Williams and Krumwiede (1924 p. 134).

1570. Omelianski's Alcohol Peptone Agar

Constituents:

1. Tap water	1000.0 cc.
2. Peptone	5.0 to 10.0 g.
3. Potassium phosphate	1.0 g.
4. Agar-agar	20.0 g.
5. Ethyl alcohol	few drops

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1. (Details of preparation not given).

Sterilization: Method not given.

Use: Study aroma producing microörganisms, *Bact. esteroaromaticum.* Author reported that more aroma was produced if little alcohol be added.

Reference: Omelianski (1923 p. 408).

1571. Robinson and Rettger's Glycerol Opsine Agar

Constituents:

1. Water	1000.0 cc.
2. Opsine	10.0 g.
3. NaCl	5.0 g.
4. Glycerol	50.0 g.

Preparation:

(1) Dissolve 2, 3, and 4 in 1 by heating.

(2) Adjust to faintly acid or basic to litmus.

(3) Boil over flame a few minutes.

(4) Filter and tube.

Sterilization: Autoclave at 12 pounds pressure for 15 minutes.

Use: General culture medium. Author reported that the medium would support the growth of some of the pathogenic forms.

Reference: Robinson and Rettger (1918 p. 212).

1572. Hesse's Glycerol Nährstoff Heyden Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Nährstoff-Heyden	5.0 g.
3. Glycerol	30.0 g.
4. Agar	10.0 g.
5. NaCl	5.0 g.

Preparation:

(1) Mix the Nährstoff Heyden with a little water in a beaker, forming a paste. Stir until all the Nährstoff-Heyden has dissolved.

(2) Dissolve 3, 4, 5 and 5.0 cc. of a normal crystal soda solution in 1 by boiling 2 hours.

(3) Mix (1) and (2).

(4) Boil for 15 minutes, stirring constantly.

(5) Filter thru folded moistened filter paper in a steamer. Five funnels may be used to hasten the process.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

References: Hess (1899 p. 505), Kolle and Wasserman (1912 p. 412), Abel (1912 p. 94), Klimmer (1923 p. 224).

1573. Molisch's Glycerol Peptone Agar

Constituents:

1. Water, Moldau	1000.0 cc.
2. Agar	18.0 g.
3. Peptone	5.0 g.
4. Glycerol	5.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of purple bacteria.

Variants: Harvey prepared Sabouraud's Glycerol Agar by dissolving 20.0 g. Chassaing's peptone, 40.0 g. glycerol and 20.0 g. agar in 1000 cc. water.

Reference: Molisch (1907 p. 11), Harvey (1921–22 p. 101).

1574. Spengler's Glycerol Somatose Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Nährstoff Heyden	5.0 g.
3. Somatose	5.0 g.
4. NaCl	5.0 g.
5. Glycerol	30.0 g.
6. Agar	15.0 g.
7. Soda	2.0 to 4.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

References: Spengler (1903 p. 92), Kolle and Wasserman (1912 p. 413).

1575. Omelianski's Formate Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Calcium formate	20.0 g.
3. Peptone	5.0 g.
4. Agar	15.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: To study decomposition of formic acid by *Bacterium formicicum*.

Variants: Author used potassium formate instead of calcium formate.

Reference: Omelianski (1903–04 pp. 185, 186).

1576. Boekhout and de Vries Tartrate Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. Sodium tartrate	5.0 g.
4. Agar	15.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of *Bacillus fuchsinus*. Author reported that after 18 hours growth was white; after 27 hours red; and a metallic sheen developed after two days.

Reference: Boekhout and de Vries (1898 p. 498).

1577. Dawson's Butter Soap Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone	2.5 g.
3. Butter soap	10.0 g.
4. Agar	20.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Culture medium, to study variation of *B. coli*.

Reference: Dawson (1919 p. 142).

1578. Harvey's Phenol Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone	30.0 g.
3. Agar	20.0 g.
4. Carbolic acid 5.0%	0.05 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Add 0.05 cc. of a 5.0% phenol solution to sterile melted (1) just before use.

Sterilization: Method not given.

Use: General culture medium.

Reference: Harvey (1921–22 p. 90).

1579. Bacto Malt Extract Agar (Synthetic) (Dehydrated)

Constituents:

1. Distilled water	
2. Maltose, technical, Difco	12.75 g.
3. Dextrin, Difco	2.75 g.
4. Glycerol, C. P.	2.35 g.
5. Peptone, Bacto	0.78 g.
6. Agar, Bacto	15.0 g.

Preparation:

(1) Dissolve 33.63 g. of Bacto Malt Extract Agar (Synthetic), (Dehydrated) in 1000.0 cc. water by boiling or better autoclaving.

Sterilization: Sterilize in the usual manner.

Use: Cultivation of yeasts and molds.

Reference: Digestive Ferments Co. (1925 p. 17).

1580. Harvey's Glucose Glycerol Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone	15.0 g.
3. Glucose	10.0 g.
4. Glycerol	5.0 g.
5. NaCl	5.0 g.
6. Agar	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Do not adjust the reaction.

Sterilization: Not specified.

Use: Cultivation of hyphomycetes. Klimmer cultivated favus and trichophyton on a similar medium.

Variants: Klimmer used 10.0 to 20.0 g. peptone and 20.0 g. of NaCl. He credited Plaust with having described this medium previously.

References: Harvey (1921–22 p. 101), Klimmer (1923 p. 228).

1581. Beijerinck's Glucose Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	20.0 g.

3. Glucose	50.0 g.
4. Peptone	20.0 g.
5. KH_2PO_4	0.5 g.
6. Ferric ammonium citrate	0.1 g.
7. Chalk	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, and 7 in 1.

(2) Pour into plates.

Sterilization: Not specified.

Use: To study pigment production by vinegar bacteria. Author reported that the medium was blackened.

Reference: Beijerinck (1911 p. 172).

1582. Sarbouraud's Glucose Peptone Agar (Serena)

Constituents:

1. Water	1000.0 cc.
2. Glucose	40.0 g.
3. Agar	18 0 g.
4. Peptone	10.0 g.
5. Tartaric acid	3.0 to 5.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of *Oidium suis*.

Reference: Serena (1913 p. 280).

1583. Chantemesse's Phenol Peptone Agar (Bezançon)

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone	30.0 g.
3. Lactose	20.0 g.
4. Agar	20.0 g.
5. Phenol	
6. Litmus	

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) When ready for use add 4 drops of a 3.0% aqueous phenol solution and 1.0 cc. of litmus solution to each 10.0 cc. sterile (1).

Use: Differentiation of colon-typhoid group. Bezançon reported that the colon colonies were red, typhoid colonies colorless.

Variants: Harvey prepared the medium as did Bezançon but added just before use, 0.05 cc. of a 5.0% phenol solution and 100.0 cc. of litmus solution to the entire lot of sterile medium. The litmus solution was prepared as follows:

(1) Grind up litmus in a mortar.

(2) Add 5 volumes 90.0% alcohol.

(3) Boil on a water bath.

(4) Decant the supernatant fluid.

(5) Add 6 parts distilled water to the residue.

(6) Boil.

(7) Allow to cool.

(8) Divide into two portions.

(9) Render one portion slightly red with dilute sulphuric acid.

(10) Add to this reddened portion the other untreated portion little by little until the mixture becomes blue again.

(11) Filter thru paper when cool.

(12) Distribute into test tubes.

(13) Sterilize at 110°C.

(14) Keep for use.

References: Bezançon (1920 p. 341), Harvey (1921–22 p. 90).

1584. Boekhout and de Vries' Maltose Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone	5.0 g.
3. Sodium tartrate	5.0 g.
4. Maltose	5.0 g.
5. Agar	15.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add 0.0, 2.0 or 5.0 g. of lactic acid or 0.0, 1.0, 2.0 or 5.0 g. NaOH to obtain a neutral acid or alkaline reaction.

Sterilization: Not specified.

Use: Cultivation of *Bacillus fuchsinus*. Author reported that the colonies were first red and then a metallic sheen developed. Organism grew only on a neutral or slightly alkaline medium.

Reference: Boekhout and de Vries (1898 p. 500).

1585. Cheyney's Maltose Peptone Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar (2.0%)	20.0 g.
3. Maltose (1.0%)	10.0 g.
4. Peptone (1.0%)	10.0 g.
5. Lactic acid	

Preparation:

(1) Dissolve 2.0% agar in distilled water.

(2) Filter thru a thin layer of cotton.

(3) Add 1.0% maltose and 1.0% peptone.

(4) Make up to exactly 2.0% acidity by the addition of lactic acid.
(5) Tube.

Sterilization: Autoclave for 10 minutes at 15 pounds pressure.

Use: Cultivation of organisms found in canned food.

Reference: Cheyney (1919 p. 183).

1586. Sabouraud's Glycerol Peptone Agar

Constituents:

1. Water	1000.0 cc.
2 Peptone	20.0 g.
3. Glycerol	20.0 g.
4. Acetic acid (glacial)	5.0 drops
5. Agar	13.0 g.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of Seborrheaie's bacilli (*Bacillus acne*).

Reference: Sabouraud (1897 p. 144).

1587. Robinson and Rettger's Glycerol Opsine Citrate Agar

Constituents:

1. Water	1000.0 cc.
2. Opsine	20.0 g.
3. NaCl	5.0 g.
4. KH_2PO_4	5.0 g.
5. Sodium citrate	2.0 g.
6. $MgSO_4$	2.0 g.
7. Glucose	5.0 g.
8. Glycerol	60.0 g.
9. Agar	15.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.
(2) Medium adjusted to very faintly alkaline to litmus.

Sterilization: Sterilize at 12 pounds for 15 minutes.

Use: General culture media. Author reported that the medium supported the growth of some pathogenic organisms.

Reference: Robinson and Rettger (1918 pp. 214, 215).

1588. Botelho's Lacto-phenol Peptone Agar

Constituents:

1. Distilled water	
2. Lactic acid	
3. Phenol (snowy)	
4. Glycerol	
5. Cotton blue ("Poirier C^{4}B")	
6. Sugar (white crystallized)	
7. NaCl	5.0 g.
8. Peptone (Chapoteaut)	10.0 g.
9. Agar	15.0 g.

Preparation:
(1) Prepare a lactophenol solution by mixing the following materials: Distilled water 1 part, glycerol 2 parts, pure lactic acid 1 part, snowy phenol 1 part.
(2) Add 0.5 centigrams of cotton blue "Poirier C^{4}B" to 30.0 g. of (1).
(3) Mix (2) and dilute to $\frac{1}{3}$ with distilled water.
(4) Mix 200.0 g. of crystalline white sugar in 100.0 cc. of cold water.
(5) Boil (4) and stir until solution is complete.
(6) Filter thru flannel.
(7) Dissolve 15.0 g. agar, 10.0 g. Chapoteaut's peptone, and 5.0 g. of NaCl in 1000.0 cc. of water by boiling.
(8) Filter, but do not neutralize.
(9) Measure 100.0 cc. of melted sterile (8) into a balloon flask.
(10) Add 2.0 cc. of (3) and 10.0 cc. of (9) and mix well.
(11) Boil.
(12) Add 7.0 cc. of a 2.0% NaOH solution in distilled water. This completely decolorizes the agar.
(13) Distribute in 3.0 cc. lots into small sterile Wassermann tubes.

Sterilization: Sterilize (8) in the autoclave.

Use: Isolation of members of colon typhoid group. Author reported that members of the colon-typhoid group color the medium blue.

Reference: Botelho (1917 p. 436).

1589. Sullivan's Ammonium Lactate Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone, Witte's	15.0 g.
3. NaCl	3.0 g.
4. K_2HPO_4	1.5 g.
5. $MgSO_4$	0.5 g.
6. $Fe_2(SO_4)_3$	trace
7. $CaCl_2$	trace
8. Ammonium lactate	0.5 g.
9. Glycerol	20.0 g.
10. Agar	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8, 9 and 10 in 1.

Sterilization: Method not given.

Use: General culture medium. Author reported that putrefactive, phosphorescent, chromogenic and pathogenic varieties developed. If the salts $CaCl_2$, $Fe_2(SO_4)_3$ and NaCl be omitted number of organisms grown was not markedly decreased.

Reference: Sullivan (1905–06 p. 113).

1590. Lipman and Brown's Nitrate Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Dextrose	10.0 g.
3. K_2HPO_4	0.5 g.
4. $MgSO_4$	0.2 g.
5. Agar	20.0 g.
6. Peptone	0.1 to 0.5 g.
7. KNO_3	0.05 to 0.5 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) To (1) containing 0.5 g. peptone, add 0.05 to 0.1, 0.2, or 0.5 g. KNO_3.

Sterilization: Method not specified.

Use: Determination of bacterial count of soil. Author reported that different soils showed different results with the same peptone and nitrate concentration.

Variants:

(a) See medium 1549.

(b) Harvey used 0.06 g. KNO_3.

References: Lipman and Brown (1910 p. 451), Harvey (1921–22 p. 105).

1591. Beijerinck's Ferric Ammonium Citrate Peptone Agar (Janke)

Constituents:

1. Water	1000.0 cc.
2. Agar	2.0 g.
3. Glucose	5.0 g.
4. Peptone	2.0 g.
5. KH_2PO_4	0.05 g.
6. Ferric ammonium citrate	0.01 g.
7. Chalk	2.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, and 7 in 1.

Sterilization: Method not given.

Use: Cultivation of acetic acid bacteria in beers.

Reference: Janke (1916 p. 6).

1592. Vierling's Nitrate Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $CaCl_2$	1.0 g.
4. $MgSO_4$	0.1 g.
5. $FeCl_3$	trace
6. NaCl	trace
7. Agar (2.0%)	20.0 g.
8. Peptone (1.0%)	10.0 g.
9. Dextrose (1.0%)	10.0 g.
10. KNO_3 (0.75%)	7.5 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1000.0 cc. water.

(2) Dissolve 2.0% agar, 1.0% peptone, 1.0% dextrose and 1.0% KNO_3 in 100.0 cc. of (1).

(3) Pour in petri dishes.

Sterilization: Method not given.

Use: To study nitrate reduction by mycobacteria. To test nitrate production, pour 2.0% KI solution over the plates which have been acidified with dilute acetic acid. If KNO_2 is formed, the iodine will be freed, coloring the starch blue. The addition of starch to the medium is not specified however.

References: Vierling (1920 p. 201).

1593. Heinemann's Asparagin Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. Asparagin	5.0 g.
4. K_2HPO_4	2.0 g.
5. Na_2HPO_4	2.0 g.
6. $MgSO_4$	2.0 g.
7. $CaCl_2$	2.0 g.
8. Ammonium lactate	2.0 g.
9. Peptone	10.0 g.
10. Starch (washed)	30.0 g.

Preparation:

(1) Dissolve agar in 600.0 cc. of water by heating.

(2) Dissolve 3, 4, 5, 6, 7 and 8 in 200.0 cc. of water. (A fine precipitate is formed).

(3) Add (2) to hot (1).

(4) Add 9.

(5) Adjust to neutral with phenolphthalein.

(6) Filter.
(7) To hot (6) gradually add a suspension of 30.0 g. washed starch made perfectly homogeneous in a mortar. Stir constantly.
(8) Bring near the boiling point and weigh. Total weight should be 1000.0 g.
(9) Tube.

Sterilization: Sterilize in autoclave at 120° for 5 minutes

Use: Substitute for potato as a culture medium. Author reported that the medium was superior to potato for the composition is always the same, reaction can be adjusted and pigment formation can be better studied.

References: Heinemann (1907 p. 283), (1922 p. 29), Besson (1920 p. 57).

1594. MacConkey's Basal Bile Salt Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Sodium glycocholate (0.5%)	5.0 g.
3. Peptone (1.5%)	15.0 g.
. Agar (1.5%)	15.0 g.

Preparation:
(1) Dissolve 2, 3, and 4 in 1.
(2) Filter.
(3) Dissolve one of the added nutrients in (2).

Sterilization: Method not specified.

Use: Differentiation *Bacillus coli commune* and *Bacillus typhi abdominalis*.

Added nutrients: The author added 0.3 to 0.5% lactose or glucose.

Variants:
(a) Frost prepared a similar medium as follows:
  (1) Dissolve 20.0 g. of agar in a liter of boiling water.
  (2) Dissolve 20.0 g. of peptone in a liter of boiling water.
  (3) Mix (1) and (2).
  (4) Make alkaline by the addition of 4.0 cc. of a normal sodium hydrate solution, after neutralizing the litmus.
  (5) While hot add 5.0 g. of sodium taurocholate, 10.0 g. of lactose and 10.0 cc. of a 5.0% watery solution of neutral red.
  (6) When the solution is complete, filter thru cotton.
  (7) Tube.
  (8) Sterilize in the steam sterilizer once for 25 or 30 minutes.
(b) MacConkey specified the use of 2.0% Witte's peptone, 0.5% commercial sodium taurocholate, added 0.5% of a 1.0% solution of neutral red, 1.0% glucose or any other carbohydrate and 0.034% $CaCl_2$, 0.5% KNO_3 or 0.5% KI might be added. The medium was clarified with white of egg before the addition of neutral red and carbohydate, and sterilized in the steamer for 10 minutes on each of two successive days. The salts, $CaCl_2$, KNO_3 and KI, in the concentrations given, stimulated the growth of lactose fermenters.
(c) MacConkey used litmus in the above variant instead of neutral red.
(d) MacConkey used 1.5 to 2.0% agar in the preparation of variant (b) and added 1.0% serum or 1.0% alkali-haematin solution, ($CaCl_2$, KNO_3 or KI were not used in this medium).
(e) Abel used 0.4% of a 1.0% neutral red solution in MacConkey's medium, variant (b) and specified the use of lactose as a carbohydrate.
(f) Ball prepared a medium containing 5.0 g. of sodium taurocholate, 15.0 g. peptone, 35.0 g. lactose and 15.0 g. of agar per liter.
(g) Percival prepared a medium as follows:
  (1) Dissolve 5.0 g. sodium taurocholate, 20.0 g. peptone, and 15.0 g. of agar in 1000.0 cc. of water by heating in a water bath for one hour.
  (2) Cool to 50°C.
  (3) Add the white of an egg and heat in the water bath for 90 minutes.
  (4) Filter.
  (5) Add 1.0 g. lactose, 1.0 cc. of a 0.5% solution of neutral red and 1.0 cc. of a 0.1% solution of crystal violet, per 100.0 cc. of the filtrate.
  (6) Tube in sterile tubes in 10.0 cc. quantities.

(7) Sterilize by heating 20 minutes on 3 successive days.

(h) Levine gave the following medium as Bile Salt (Rebipel) Agar (After Savage): Sodium taurocholate 5 grams, Witte's peptone 20 grams and distilled water 1 liter, are boiled up together, 20 grams of agar are added and dissolved in the solution in the autoclave in the ordinary way. The medium is cleared with white of egg and filtered. After filtration, 10 grams of lactose and 5 cc. of recently prepared 1 per cent neutral red solution are added. The medium is then tubed and sterilized for 15 minutes on three successive days.

(i) Harvey gave the following method of preparation:

(1) Dissolve 20.0 g. peptone, 5.0 g. sodium taurocholate in 1000.0 cc. tap water and make faintly alkaline to litmus.

(2) Steam 45 minutes.

(3) Add 15.0 g. powdered agar, making it into a paste or suspension, before addition, with a little of the taurocholate peptone solution.

(4) Steam gently 2½ hours to bring the agar thoroughly into solution.

(5) Bring the volume up to 1000.0 cc. by the addition of water.

(6) Cool to 60°C., clarify by the addition of egg and filter.

(7) Dissolve 10.0 g. lactose (or other sugar if desired) in 15.0 cc. sterile water and steam 15 minutes.

(8) Add the lactose solution to the hot, clear, nutrient agar.

(9) Add 5.0 cc. freshly prepared sterile 1 per cent neutral red by means of a sterile pipette.

(10) Distribute the resulting agar, which is deep red, into flasks or test tubes.

(11) Steam 25 minutes.

(j) Cunningham prepared the medium as follows:

(1) Steam 5.0 g. sodium taurocholate and 20.0 g. peptone in 1000.0 cc. water for one hour.

(2) Filter.

(3) Add 1.5% agar and steam for one hour.

(4) Filter while hot thru cotton wool.

(5) Add 1.0% lactose dissolved in a little water and 1.0% Andrades indicator.

(6) Sterilize intermittently.

References: MacConkey (1900 p. 20), (1901 p. 740), Frost (1903 p. 342), MacConkey (1905 pp. 334, 336), (1908 p. 325), Abel (1912 p. 227), Ball (1919 p. 81), Tanner (1919 p. 49), Percival (1920 p. 307), Harvey (1921–22 p. 89), Klimmer (1923 p. 214), Levine (1921 p. 116), Cunningham (1924 p. 98).

1595. Harrison and VanderLeck's Aesculin Bile Salt Agar

Constituents:

1. Water...............	1000.0 cc.
2. Peptone (Witte (1.0 or 2.0%)...............	10.0 or 20.0 g.
3. Sodium taurocholate (Commercial) (0.5%)	5.0 g.
4. Aesculin (0.1%)......	1.0 g.
5. Iron citrate (0.05%).	0.5 g.
6. Agar................	15.0 g.

Preparation:

(1) Dissolve the agar in part of 1.

(2) Add 2, 3 and 4 to the rest of 1.

(3) Mix (1) and (2).

(4) Boil and filter (may be clarified with egg white).

(5) Tube.

Sterilization: Sterilize by steaming on 3 successive days or autoclaving at 15 pounds for 15 minutes.

Use: Presumptive test for *B. coli* in water analysis. Author reported that *B. coli* colonies were black, typhoid colonies produced no blackening.

Variants:

(a) The authors used 0.25% commercial bile salt, 1.0% Witte's peptone and 0.1% iron citrate instead of amounts used above.

(b) Levine gave Eyre's method of preparation as follows:

(1) Measure out 400.0 cc. distilled water into a tared 2 liter flask.

(2) Weigh out 15 grams agar, 10 grams peptone, 5 grams sodium taurocholate and make into a thick paste with 150.0 cc. distilled water.

(3) Add this paste to the distilled water in the flask.

(4) Dissolve the ingredients by bubbling live steam thru the mixture.

(5) Weigh out 1.0 g. aesculin and 0.5 g. ferric citrate and dissolve in a second flask containing 100.0 cc. distilled water.

(6) Mix the contents of the two flasks—adjust the weight to the calculated medium figure (in this case 1031. 5 g.) by the addition of distilled water at 100°C.

(7) Clarify with egg and filter.

(8) Tube and sterilize as for nutrient agar.

References: Harrison and VanderLeck (1909 p. 549, 607), Committee Am. Public Health Asso. (1909 p. 286), Harvey (1921–22 p. 89), Levine (1921 p. 117), Heinemann (1922 p. 33).

1596. Harvey's Brilliant Green Bile Salt Agar

Constituents:

1. Water	1000.0 cc.
2. Sodium taurocholate	5.0 g.
3. Peptone	30.0 g.
4. NaCl	5.0 g.
5. Lactose	10.0 g.
6. Powdered agar	30.0 g.
7. 1–1000 Brilliant-green	20.0 cc.
8. Picric acid (1.0%)	20.0 cc.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1000.0 cc. of water.

(2) Reaction to be 1.5% acid to phenolphthalein.

Sterilization: Method not given.

Use: Enrichment medium for colon-typhoid group.

Reference: Harvey (1921–22 p. 91).

SUBGROUP II-C. SECTION 4

Basal or complete media containing agar and peptone (or other commercial digest) together with additional constituents of unknown chemical composition of plant or soil origin.

A_1. Containing yeast or yeast derivatives.

B_1. All additional constituents of known chemical composition.

Gassner's Yeast Extract Peptone Agar 1597
Cohen and Clark's Yeast Extract Peptone Agar 1598
Ickert's Yeast Infusion Peptone Agar 1599
Sherman's Yeast Extract Peptone Agar 1600

B_2. Containing additional constituents of unknown chemical composition.

Rivers and Kohn's Basal Yeast Extract Peptone Agar 1601
Sturtevant's Egg Yolk Yeast Agar. 1602

A_2. Containing bacterial or fungus derivatives.

Savini and Savini-Castano's Hemoglobin Bacterial Extract Agar.... 1603
Mankowski's Fungus Infusion Peptone Agar 1604

A_3. Containing derivatives of plants other than A_1 and A_2 above.

B_1. Plant juices or tissues (not infusions) employed.

Owens' Cane Juice Peptone Agar... 1605
Plaisance and Hammer's Corn Juice Peptone Agar 1606
Peglion's Grape Must Peptone Agar 1607
Harvey's Banana Agar 1608

B_2. Plant infusions, extracts, etc., employed.

C_1. Grain derivatives used.

Meacham et al. Malt Extract Agar.. 1609
Marpmann's Flour Casein Agar.... 1610
Williams and Povitzky's Flour Peptone Agar 1611
Otabe's Wheat Peptone Agar....... 1612
Dawson's Flour Peptone Agar...... 1613
Plaisance and Hammer's Stover Infusion Peptone Agar 1614

C_2. Potato derivatives used.

De Gaetano's Potato Extract Agar.. 1615
Jochmann's Potato Bouillon Agar.. 1616
Nicholle and Alilaire's Potato Infusion Agar 1617
Shiga et al. Potato Blood Agar...... 1618
Gaehtgen's Potato Peptone Agar... 1619

C_3. Derivatives of legumes used.

Matzuschita's Pea Bile Agar....... 1620
Tanner's Pea Extract Tryp. Agar... 1621
Behrens' Pea Blood Agar.......... 1622
De Rossi's *V. Faba* Infusion Peptone Agar 1623

C_4. Miscellaneous plant derivatives used.

Owens' Molasses Peptone Agar..... 1624
Seiffert and Bamberger's Chlorophyll Bouillon Agar 1625
Dawson's Edestin Peptone Agar.... 1626

A_4. Containing soil or its derivatives.
Temple's Soil Infusion Peptone Agar........................... 1627

1597. Gassner's Yeast Extract Peptone Agar

Constituents:

1.	Water	900.0 cc.
2	Yeast extract water	900.0 cc.
3.	Agar	30.0 g.
4.	NaCl	5.0 g.
5.	Peptone	20.0 g.

Preparation:
(1) Exact method of yeast extract water not given.
(2) Mix 1 and (1).
(3) Dissolve 3 and 4.
(4) Adjust to slightly alkaline to litmus.
(5) Dissolve 5 in (4).
(6) To each 80.0 cc. of (5) add 20.0 cc. of a 0.5% solution of water blue and 0.5, 0.1 or 1.0% dextrose.

Sterilization: Not specified.

Use: To study nitrogen requirements of colon-typhoid and dysentery group.

Variants: The author omitted the glucose.

Reference: Gassner (1917–18 p. 260).

1598. Cohen and Clark's Yeast Extract Peptone Agar

Constituents:

1.	Water	1000.0 cc.
2.	Yeast, Brewer's	10.0 g.
3.	Peptone, Difco	10.0 g.
4.	K_2HPO_4	5.0 g.
5.	Agar (amount not given)	
6.	Glucose	10.0 g.

Preparation:
(1) Dissolve 5 in an extract of 10.0 g. of Brewer's yeast in 1000.0 cc. water containing 3, 4 and 6.
(2) Filter.
(3) Adjust with NaOH and HCl to desired pH.

Sterilization: Method not given.

Use: To study growth of *B. vulgaricus* and *B. coli* at different H-ion concentrations.

Reference: Cohen and Clark (1919 p. 421).

1599. Ickert's Yeast Infusion Peptone Agar

Same as medium 695 but solidified by the addition of agar.

1600. Sherman's Yeast Extract Peptone Agar

Constituents:

1.	Water	1000.0 cc.
2.	Peptone	20.0 g.
3.	Yeast	10.0 g.
4.	Lactic acid (as sodium lactate)	10.0 g.
5.	Agar	1.5 g.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Details of method of preparation not given.

Sterilization: Method not given.

Use: Isolation of organisms causing flavors in cheese.

Reference: Sherman (1921 p. 388).

1601. Rivers and Kohn's Basal Yeast Extract Peptone Agar

Constituents:

1.	Distilled water	1000.0 cc.
2.	Peptone	20 0 g.
3.	Agar agar	15.0 g.
4.	NaCl	5 0 g.
5.	Blood clot extract	
6.	Yeast extract	
7.	Brom Cresol Purple (25 0% alcoholic soln.)	

Preparation:
(1) Dissolve 2, 3 and 4 in 1 by boiling
(2) Adjust the reaction to pH = 7.4.
(3) Filter.
(4) To 15.0 cc. of sterile (3) add 20.0 cc. of sterile blood clot extract (method of preparation not given) and 15 to 20.0 cc. of yeast extract (preparation not given).
(5) Add 10.0 cc. of a 10.0% solution of any one of the added nutrients, and 25.0% alcoholic solution of brom cresol purple to give a good color.
(6) Incubate to test sterility.

Sterilization: Sterilize (3) and the blood extract in autoclave. Sterilization of other materials not given.

Use: To study fermentation ability of influenza bacillus producing meningitis.

Added nutrients: The authors added 10.0 cc. of a 10.0% solution of any desired carbohydrate, alcohol, etc., to each 100.0 cc. of medium.

Reference: Rivers and Kohn (1921 p. 381).

1602. Sturtevant's Egg Yolk Yeast Agar

Constituents:

1. Water	1000.0 cc.
2. Yeast (dry)	10.0 g.
3. Peptone	10.0 g.
4. Sodium glycero-phosphate (buffer)	5.0 g.
5. Agar	15.0 g.
6. Egg yolk	

Preparation:

(1) Heat 10.0 g. dry yeast, 10.0 g. peptone and 5.0 g. buffer (sodium glycero-phosphate) in 500.0 cc. of water with flowing steam for 30 minutes.
(2) Adjust to pH = 7.6 to 7.8.
(3) Boil for one minute over a free flame.
(4) Filter thru paper on a perforated porcelain funnel, using siliceous earth to clarify.
(5) Place 15.0 g. of shred agar in 1000.0 cc. distilled water and allow to stand for 24 hours at room temperature.
(6) Pour off as much of the water as possible by placing a piece of cheese cloth over the top of the flask.
(7) Add as much distilled water as was poured off and soak for another 24 hours.
(8) Filter thru a cotton flannel cloth, and wash the agar with 500.0 cc. of distilled water.
(9) Allow to drain, and squeeze the agar as free from water as possible.
(10) Add enough water to (9) so that the total weight will be 515.0 g.
(11) Dissolve the agar in the water by heating.
(12) Filter.
(13) Mix equal parts of (4) and (12) (double strength agar).
(14) Prepare a series of flasks containing 200.0 cc. of water. 0.5 to 1.0% neutral buffer salt may be added to keep the acidity down which develops on long standing. A small amount of normal NaOH 2 to 3.0 cc. is added. This tends to keep the egg yolk suspension at the desired pH. Plug the flasks with cotton and protect the plug with a paper cap.
(15) Add under aseptic conditions the yolk of one egg to each sterile flask of (14). Mix well.
(16) Adjust the reaction of (13) so that upon the addition of 1.0 cc. of (15) to 10.0 cc. of the agar, the final pH value will be about 6.8.
(17) Tube the agar (without the addition of egg yolk suspension) in 10.0 cc. lots.
(18) When desired for use add 1.0 to 2.0 cc. of (15) to each tube of melted agar under aseptic conditions, mix well and slant. The agar should be cooled to about 55°C.

Sterilization: Sterilize (14); method not given. Sterilize (17) by autoclaving at 15 pounds pressure for 15 minutes.

Use: Isolation of organism causing American foul brood, *Bacillus larvae*. To isolate the organism, drop some infected material into the water of condensation and smear over the surface of the agar. Author reported that best growth was obtained when 0.5% glucose was added. To make counts, add only 10 or 15 drops of the egg yolk suspension, or 1.0 cc. of the supernatant fluid of the suspension to 10.0 cc. yeast extract agar containing varying amounts of glucose. Mix thoroly and pour in plates. Add a dilute solution of fuchsin to the plates when counting. This colors the colonies.

Variants: Author added from 0.5 to 10.0% glucose.

Reference: Sturtevant (1924 p. 136).

1603. Savini and Savini-Gastano's Hemoglobin Bacterial Extract Agar

Same as medium 977 but agar employed instead of bouillon.

1604. Mankowski's Fungus Infusion Peptone Agar

Constituents:

1. Fungus infusion	1000.0 cc.
2. Agar	15 0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.

Preparation:

(1) Method of preparation of Fungus infusion not given.
(2) Dissolve 2, 3 and 4 in (1).
(3) Clarify with egg white.
(4) The reaction is neutral.

Sterilization: Not specified.

Use: Differentiation of typhoid bacilli and *Bacterium coli communis*. Author reported that *Bacterium coli* grew in a silver white, solid and dry film. Typhoid bacilli grew slower, appeared as transparent, shining, damp strips. When fuchsin indigo carmine be added typhoid bacilli colored the blue medium red, *Bacterium coli communis* gave a bluish green color and finally completely decolorized the medium.

Variants:

(a) The author prepared a medium containing fuchsin and indigo carmine by continuing from step (4) above as follows:

- (5) Prepare a saturated solution of acid fuchsin in a 1.0% KOH solution (acid fuchsin may be added to a 1.0% KOH solution until a dark black brown color is reached).
- (6) Prepare a watery saturated solution of indigo carmine.
- (7) Add 2.0 cc. of (2) and 1.0 cc. of (3) to 22.0 cc. of distilled water. This solution is dark blue and reaction slightly alkaline.
- (8) Add (7) drop by drop to (4) until the agar is colored blue and then violet blue.
- (9) Distribute into test tubes.
- (10) Add to each test tube a drop of watery saturated solution of indigo carmine.

(b) Wiegert solidified medium 701 by the addition of agar.

References: Mankowski (1900 p. 23), Wiegert (1921–22 p. 110).

1605. Owens' Cane Juice Peptone Agar

Constituents:

1. Cane juice	1000.0 cc.
2. Agar (2.0%)	20.0 g.
3. Peptone (1.0%)	10.0 g.

Preparation:

(1) Heat fresh raw cane juice.

(2) Filter thru cotton.

(3) Add 1.0% peptone (peptone may be omitted).

(4) Solidify by the addition of 2.0% agar.

Sterilization: Not specified.

Use: Bacterial count of cane sugar products.

Reference: Owen (1914 p. 337).

1606. Plaisance and Hammer's Corn Juice Peptone Agar

Constituents:

1. Corn juice	1000.0 cc.
2. Agar	15.0 g.
3. Peptone	10.0 g.

Preparation:

(1) Obtain corn juice by pressing green corn.

(2) Dissolve 2 and 3 in (1).

(3) Adjustment of reaction not specified.

Sterilization: Not specified.

Use: Study mannitol producing organisms from silage.

Reference: Plaisance and Hammer (1921 p. 432).

1607. Peglion's Grape Must Peptone Agar

Constituents:

1. Grape must	500.0 cc.
2. Peptone	10.0 g.
3. Ammonium phosphate	2.0 g.
4. Glycerol	10.0 cc.
5. Agar	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Pour into petri dishes or plates.

Sterilization: Not specified.

Use: Cultivation of *Saccharomyces ellipsoideus*.

Reference: Peglion (1898 p. 477).

1608. Harvey's Banana Agar

Constituents:

1. Agar (nutrient)	1000.0 cc.
2. Banana (10.0%)	100.0 g.

Preparation:

(1) Use banana in the form of cut cylinder or as 10.0% pulp incorporated with nutrient agar. (Medium 779 variant (bb) solidified by the addition of agar.)

Sterilization: Not specified.

Use: General culture medium.

Reference: Harvey (1921–22 p. 119).

1609. Meacham et al. Malt Extract Agar

Constituents:

1. Malt extract (2.5%) agar	1000.0 cc.
2. K_2HPO_4 M/50	
3. Acetic acid N/50	

Preparation:

(1) Preparation of malt extract agar not given.

(2) Dissolve 2 and 4 in (1).
(3) Reaction about pH = 5.7.

Sterilization: Method not given.

Use: To study phosphate-phthalate and phosphate-acetate as buffers for cultivation of *E. parasitica* (Murr). Author reported that acid phthalate gave best growth of *E. parasitica.*

Variants: Author used M/50 acid potassium phthalate instead of acetic acid.

Reference: Meacham, Hopfield and Acree (1920 p. 305).

1610. Marpmann's Flour Casein Agar

Constituents:

1. Bouillon	1000.0 cc.
2. Glycerol	30.0 g.
3. Casein, dissolved	50.0 g.
4. Sodium phosphate	3.0 g.
5. Rye flour	50.0 g.
6. Gelatin	40.0 g.
7. Agar	3.0 g.

Preparation:

(1) Method of preparation or exact composition of bouillon not given.
(2) Dissolve 2, 3, 4, 5, 6 and 7 in (1).
(3) Adjustment of reaction not given.
(4) Distribute into flat bottomed flasks.

Sterilization: Method not given.

Use: Cultivation of tubercle bacilli.

Reference: Marpmann (1903 p. 636).

1611. Williams and Povitzky's Flour Peptone Agar

Constituents:

1. Water (tap)	1300.0 cc.
2. Wheat	1.0 lb.
3. Diastase	0.5 g.
4. NaCl	5.0 g.
5. Peptone	10.0 g.
6. Agar (1.5%)	15.0 g.
7. Gelatin (2.0%)	20.0 g.

Preparation:

(1) Roast whole wheat (with or without the husk) in pan until brown.
(2) To one pound roasted wheat add 1300.0 cc. tap water.
(3) Steam in Arnold for one-half hour.
(4) Strain thru fine wire strainer.
(5) Make up to 1000.0 cc. with tap water.
(6) Cool to 35°C. and add Taka diastase or ordinary diastase, 0.5 gm. and shake well. Keep flask at 30–40°C. for half hour.
(7) Add sodium chloride, 5 g. and peptone 10.0 g.
(8) Boil. Reaction adjusted to pH-7.4.
(9) Add agar 1.5% and autoclave at 15 pounds pressure for half hour.
(10) On removal from autoclave add gelatin 2.0%. Adjust reaction.
(11) Cool down to 45°C. and clarify with white of egg.
(12) Heat in the Arnold steamer 45 minutes. Correct reaction to pH-7.6. This will give desired end reaction (pH-7.4), after autoclaving.
(13) Filter thru thin cotton (3 times) and tube.

Sterilization: Sterilize the tubes of agar for 30 minutes at 15 pounds pressure.

Use: Cultivation of *B. influenzae.* Author reported that growth of the influenza organism was obtained when mixed with other organisms.

Reference: Williams and Povitzky (1920–21 p. 407).

1612. Otabe's Wheat Peptone Agar

Solidify medium 707 by the addition of 1.5 to 2.0% agar.

1613. Dawson's Flour Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone	2.5 g.
3. Flour protein	10.0 g.
4. Agar	20.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Reaction to be alkaline.

Sterilization: Method not given.

Use: Culture medium to study variation of *B. coli.*

Reference: Dawson (1919 p. 142).

1614. Plaisance and Hammer's Stover Infusion Peptone Agar

Constituents:

1. Stover juice	1000.0 cc.
2. Agar	15.0 g.
3. Peptone	10.0 g.
4. Sucrose	5.0 g.

Preparation:

(1) Prepare stover juice by soaking corn stover in water for 12 hours and then pressing.
(2) Dissolve 2, 3 and 4 in (1).

(3) May be cleared with egg.

Sterilization: Not specified.

Use: Study mannitol producing organisms from silage. Authors reported this medium to be inferior to 1606.

Reference: Plaisance and Hammer (1921 p. 432).

1615. De Gaetano's Potato Extract Agar

Constituents:

1. Potato infusion	1000.0 cc.
2. Meat extract (Liebig's)	20.0 g.
3. Peptone	10.0 g.
4. Agar	20.0 to 30.0 g.
5. Tartaric acid	
6. Glucose	

Preparation:

(1) Preparation of potato infusion not given.
(2) Dissolve 2, 3, and 4 in (1) by boiling.
(3) Filter.
(4) Distribute in 6.0 cc. lots in tubes.
(5) To each tube add 5.0 to 6.0 drops of 10.0 to 15.0% tartaric acid solution and 5.0 to 6.0 drops of a 50.0% glucose solution.

Sterilization: Method not given.

Use: Cultivation of *Saccharomyces septicus*.

References: De Gaetano (1897 #200), Kamen (1899 p. 833).

1616. Jochmann's Potato Bouillon Agar

Constituents:

1. Bouillon	200.0 cc.
2. Potato	600.0 g.
3. Agar	3.75 g.

Preparation:

(1) Grate 600.0 g. of potatoes and allow to stand in a glass flask at about 15°C. for 12 hours.
(2) Press the juice thru a filter cloth using the hands to expell all the juice.
(3) To 300.0 g. of (2) add 200.0 g. of weakly alkaline bouillon (exact composition or method of preparation not given).
(4) Dissolve completely 3.75 g. finely divided agar in (3) using the autoclave.
(5) The reaction is slightly acid.

Sterilization: Not specified.

Use: Diagnosis of typhoid fever. Author reported that typhoid colonies after 12 hours were typical, small, silver gray, glistening, fine fibered; coli colonies larger, light yellow and mostly all round. Medium was dark yellow or dark brown in color.

Reference: Jochmann (1902 p. 461).

1617. Nicolle and Alilaire's Potato Infusion Agar

Constituents:

1. Water	2000.0 cc.
2. Potato	500.0 g.
3. Beef	500.0 g.
4. Peptone (Chapoteaut)	30.0 g.
5. NaCl	10.0 g.
6. Glycerol	20.0 g.
7. Agar	60.0 g.

Preparation:

(1) Extract 500.0 g. of finely chopped beef with one liter of water over night.
(2) Extract 500.0 g. of potatoes cut in large pieces in a second liter of water over night.
(3) Mix (1) and (2).
(4) Dissolve 4, 5, 6 and 7 in (3).
(5) Proceed in the ordinary manner for the rest of the preparation for nutrient agar. (Method not given).

Sterilization: Not specified.

Use: Mass cultivation of *B. encapsulas* and Cholera vibrio.

Variants: Lubinski solidified medium 873 by the addition of 1.0 to 1.5% agar.

References: Nicolle and Alilaire (1909 p. 548), Lubinski (1895 p. 126).

1618. Shiga et al Potato Blood Agar

Constituents:

1. Glycerol (4.0% soln)	250.0 cc.
2. Potato	125.0 g.
3. Meat water	750.0 cc.
4. Agar	20.0 g.
5. Peptone	10.0 g.
6. NaCl	5.0 g.
7. Blood (beef or horse)	4000.0 cc.

Preparation:

(1) Peel 500.0 g. of potatoes and cut into small pieces.
(2) Add (1) to 1000.0 cc. of 4.0% glycerol water.
(3) Boil for 30 minutes in the autoclave.
(4) Filter thru gauze.

(5) Method of preparation of meat water not given.
(6) Mix 250.0 cc. of (4) and 750.0 cc. of (5).
(7) Add 20.0 g. of agar.
(8) Boil in the autoclave for one hour.
(9) Make slightly alkaline.
(10) Filter.
(11) Add 1.0% Witte's peptone and 0.5% NaCl.
(12) Distribute into tubes.
(13) Melt and cool to 50°C. when ready for use.
(14) Add agar to beef or horse blood in the ration of 1:4.
(15) Tube and slant or pour into petri dishes.
(16) After solidification heat the agar at 56°C. for 30 minutes.

Sterilization: Not specified.

Use: Cultivation of whooping cough bacilli and influenza bacilli.

Reference: Shiga, Imai and Eguchi (1913 p. 104).

1619. Gaehtgen's Potato Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Potato	500.0 g.
3. Agar	
4. Peptone	
5. NaCl	

Preparation:

(1) Carefully wash and peel 500.0 g. potatoes.
(2) Grind the potatoes fine by means of a porcelain mortar. This is to be carried out under the water as far as possible.
(3) Boil for one hour.
(4) Dissolve 3, 4 and 5 (amounts not given) in the paste.
(5) Add soda to obtain the desired reaction.

Sterilization: Method not given.

Use: Inexpensive culture Medium. Author reported that the medium was not transparent.

Reference: Gaehtgens (1916 p. 47).

1620. Matzuschita's Pea Bile Agar

Constituents:

1. Water	1000.0 cc.
2. Liver, ox	500.0 g.
3. Pea flour	30.0 g.
4. Peptone (0.7%)	7.0 g.
5. NaCl (0.5%)	5.0 g.
6. Bile, ox	600.0 g.
7. Agar (2.0%)	32.0 g.

Preparation:

(1) Boil 500.0 g. of finely chopped liver with 30.0 g. of pea flour and one liter of water.
(2) Allow to cool and add 0.5% peptone, 0.5% NaCl and 0.02% HCl.
(3) Mix thoroly and allow to stand for 3 hours at 37°C.
(4) Add 600.0 g. of ox bile and allow to stand another 3 hours at 37°C.
(5) Proceed as in the preparation of regular nutrient agar by boiling, filtering, adding 2.0% agar and filtering again.
(6) Reaction is slightly acid.

Sterilization: Method not given.

Use: Cultivation of intestinal bacteria.

Reference: Matzuschita (1901–02 p. 214).

1621. Tanner's Pea Extract Tryp. Agar

Constituents:

1. Medium 1116	1000.0 cc.
2. Agar (2.0%)	20.0 g.
3. $CaCl_2$	0.125 g.
4. Pea extract (5.0%)	50.0 cc.

Preparation:

(1) Add 2.0% agar fiber and 0.125 g. of $CaCl_2$ to 1000.0 cc. of trypsinized broth, medium 1116.
(2) Autoclave at 118°C. for 45 minutes to dissolve the agar.
(3) Mix together in a sauce pan.
(4) Titrate with N/10 KOH to give an absolutely neutral reaction.
(5) Cool to 60°C.
(6) Add the whites of two eggs beaten up with the crushed shells.
(7) Autoclave again at 118°C. for 75 minutes (or in the steamer for two hours).
(8) Filter.
(9) Add 5.0% sterile pea extract (preparation not given).

Sterilization: Sterilize in the ordinary way (method not given).

Use: General culture medium.

Reference: Tanner (1919 p. 49).

1622. Behrens' Pea Blood Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Peas	10.0 g.
3. Peptone	20.0 g.
4. NaCl	5.0 g.
5. $CaCl_2$	0.1 g.
6. N/1 Na_2CO_3 soln	10.0 cc.
7. Agar	20.0 g.
8. Blood (rabbit)	2000.0 cc.

Preparation:

(1) Boil 10.0 g. peas in 1000.0 cc. distilled water to obtain extract. Length of boiling not specified.
(2) Strain, boil the extract and filter.
(3) Dialyze the filtrate in a large collodium sac against running distilled water for 24 to 48 hours.
(4) Dilute (3) to 1000.0 cc.
(5) Dissolve 3, 4, 5, 6 and 7 in (4).
(6) Distribute in test tubes in 1.0 cc. lots.
(7) Shortly before use the desired number of sterile agar tubes are melted in the water bath, cooled to 60°C. and two volumes of defibrinated rabbit's blood are added.
(8) Mix well and solidify in slanting position.

Sterilization: Sterilize by autoclaving at 105 to 108°C. for 15 minutes.

Use: Special culture medium for *Trypanosoma Brucei*.

Variants: The author used beans instead of peas.

Reference: Behrens (1914 p. 28).

1623. De'Rossi's *V. Faba* Infusion Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. *V. Faba* leaves	100.0 g.
3. Agar (1.5%)	15.0 g.
4. NaCl	5.0 g.
5. Sucrose (2.0%)	20.0 g.
6. Peptone (1.0%)	10.0 g.

Preparation:

(1) Boil 8 to 10 parts water with *Vicia Faba* leaves.
(2) Filter and add 0.5% NaCl, 2.0% sucrose and 1.0% peptone. (NaCl, sucrose and peptone may be omitted.)
(3) Continue in the same manner as in the preparation of nutrient agar.
(4) The reaction is slightly acid or alkaline.

Sterilization: Not specified.

Use: Cultivation of bacteria found in the nodules of leguminous plants.

Variants: The author used 2.0% glucose instead of sucrose.

Reference: de'Rossi (1907 p. 301).

1624. Owen's Molasses Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Molasses (Final 75 Brix)	160.0 g.
3. Agar	20.0 g.
4. Peptone	10.0 g.

Preparation:

(1) Dissolve 160.0 g. of final molasses, 75 Brix, 20.0 g. agar and 10.0 g. peptone in 1000.0 cc. water.

Sterilization: Not specified.

Use: Bacterial counts of organisms found in corn juice and cane sugar products.

Reference: Owen (1914 p. 338).

1625. Seiffert and Bamberger's Chlorophyll Bouillon Agar

Constituents:

1. Neutral agar	1000.0 cc.
2. Chlorophyll solution ("Solutio spirituosa," Merck)	25.0 cc.
3. Sucrose	
4. Dextrin	
5. Diamond fuchsin solution	
6. Na_2SO_3	
7. Soda	

Preparation:

(1) Prepare 60.0 cc. of a 10.0% soda solution using water free soda.
(2) Add 25.0 cc. chlorophyll solution (Solutio spirituose Merck) to (1).
(3) Heat for one hour in the steamer.
(4) Prepare a 20.0% sucrose solution.
(5) Prepare a 20.0% dextrin solution.
(6) Add 50.0 cc. sterile (4) and 50.0 cc. sterile (5) to (3).
(7) Mix with one liter sterile neutral agar.
(8) Prepare a diamond fuchsin adding an excess of diamond fuchsin to absolute alcohol and incubating for 24 hours. Shake often.
(9) Add 4.0 cc. of (8) to (7) when ready for use.

(10) Add about 15.0 cc. of a 10.0% sterile Na_2SO_3 solution to decolorize (9).
(11) Pour in sterile plates.
(12) Dry the plates in the air.

Sterilization: Method of sterilization of (4), (5) or neutral agar not given.

Use: Selective medium for cholera. Author reported that the cholera colonies were red. Other organisms were inhibited.

Reference: Seiffert and Bamberger (1916 p. 288).

1626. Dawson's Edestin Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone	5.0 g.
3. Edestin	5.0 g.
4. Agar	20.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Reaction to be alkaline.

Sterilization: Method not given.

Use: Culture medium to study variation of *B. coli*.

Reference: Dawson (1919 p. 142).

1627. Temple's Soil Infusion Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Soil	100.0 g.
3. K_2HPO_4	1.0 g.
4. Peptone	10.0 g.
5. Agar	15.0 g.

Preparation:

(1) Prepare a soil infusion by adding 1000.0 cc of water to 100.0 g. soil and bringing to a boil.
(2) Filter.
(3) Dissolve 3, 4 and 5 in (2).
(4) Adjust the reaction to +0.5.

Sterilization: Not specified.

Use: Determination of bacterial counts from soil.

Variants: Brown suggested the use of 0.05 g. peptone instead of 10.0 g.

References: Temple (1912 p. 205), Brown (1913 p. 499).

SUBGROUP II-C. SECTION 5

Basal or complete media containing agar and peptone (or other commercial digest) and at least one other organic material of unknown composition.

A_1. Animal cells and tissues or their derivatives added.

Rivers and Kohn's Basal Blood Cell Peptone Agar	1628
Krumwiede, Pratt and Grund's Egg Peptone Agar	1629
Weiss' Nährstoff Heyden Gelatin Agar	1630
Deycke's Alkaline Albumin Gelatin Agar	1631
Deycke's Albuminate Glycerol Agar	1631a
Krumwiede and Pratt's Gelatin Serum Agar	1632
Dunschmann's Bile Salt Peptone Agar (Bezançon)	1633
Capaldi's Peptone Gelatin Agar	1634
Smyth's Egg Trypsinized Peptone Agar	1635

A_2. Animal fluids added.

B_1. Blood employed.

Czaplewski's Blood Nährstoff Heyden Agar (Klimmer)	1636
Norris et al. Blood Peptone Agar	1637
Nicolle's Blood Peptone Agar (Behrens)	1638
Kelser's Blood Peptone Agar	1639
Harvey's Blood Peptone Agar	1640
Fildes' Blood Peptone Agar	1641
Golowkoff's Blood Peptone Agar (Uche)	1642
Harvey's Blood Serum Peptone Agar	1643
Harvey's Litmus Blood Peptone Agar	1644

B_2. Serum or ascitic fluid employed.

Tochtermann's Serum Peptone Agar	1645
Browning's Telluric Acid Serum Agar (Wood)	1646
Teague and Deibert's Serum Peptone Agar	1647
Emile-Weil's Fleuritic Serum Peptone Agar	1648

B_3. Bile or its derivatives employed.

Bacto MacConkey's Agar (Dehydrated)	1649
Jordan's Basal Peptone Bile Agar	1650
Padlewsky's Bile Peptone Agar (Bezançon)	1651
Northrup's Peptone Bile Agar	1652

A_3. Animal secretions, excretions or their derivatives added.

Bacto Whey Agar (Dehydrated)	1653
Sabouraud and Noire's Basal Whey Peptone Agar (Weil & Noire)	1654
Tanner's Whey Peptone Agar	1655
Committee A. P. H. A. Whey Peptone Agar	1656
Huss' Whey Peptone Agar	1657
Heller's Peptone Urine Agar	1658
Loeffler's Malachite Green Nutrose Peptone Agar	1659
Raskin's Whey Peptone Agar	1660

1628. Rivers and Kohn's Basic Blood Cell Peptone Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Fairchild)	20.0 g.
3. NaCl	5.0 g.
4. Agar	15.0 g.
5. Red Blood Cells	10.0 cc.
6. Brom cresol purple (25.0% alc. soln.)	

Preparation:

(1) Dissolve 2, 3 and 4 in 1 by boiling.
(2) Titrate to pH = 7.4.
(3) Filter and distribute in 100.0 cc. lots.
(4) At the time the agar is taken from the autoclave while still at 95°C. add 1.0 cc. of washed red blood cells, 10.0 cc. of a 10.0% solution of one of the added nutrients and enough 25.0% alcoholic solution of brom cresol purple to each 100.0 cc. to give a good color.
(5) Tube while still warm.
(6) Incubate to test sterility.

Sterilization: Sterilize (3) in the autoclave.

Use: To study fermentation. Authors reported that the medium had a dirty looking appearance but this did not interfere with the results.

Added nutrients: The authors added 10.0 cc. of a 10.0% solution of one of the following to each 100.0 cc. of agar:

glucose
galactose
xylose
fructose
maltose

Reference: Rivers & Kohn (1921 p. 481).

1629. Krumwiede, Pratt and Grund's Egg Peptone Agar

Constituents:

1. Water	850.0 cc.
2. Whole egg	150.0 cc.
3. Na_2CO_3	3.6 to 4.05 g.
4. Peptone	7.0 g.
5. NaCl	3.5 g.
6. Agar	21.0 g.

Preparation:

(1) Mix 150.0 cc. water and whole eggs in equal parts, add 3 and shake thoroughly. (May be filtered thru thin layer of cotton to remove any thick part of egg.)
(2) Steam (1) for 20 minutes.
(3) Dissolve 4, 5 and 6 in 700.0 cc. of water.
(4) Adjustment of reaction of (3) not specified.
(5) Mix (2) and (3) while (3) is boiling hot.
(6) Pour medium thick plates and allow to stand open for 20 to 30 minutes.

Sterilization: Method not given.

Use: Isolation of cholera vibrio. Author reported that colonies by transmitted light appeared to be deep in agar, with hazy appearance.

References: Krumwiede, Pratt and Grund (1912 p. 137), Stitt (1923 p. 50).

1630. Weiss' Nährstoff Heyden Gelatin Agar

Constituents:

1. Water	1000.0 cc.
2. Gelatin	75.0 g.
3. Nährstoff Heyden	4.0 g.
4. Agar	7.5 g.

Preparation:

(1) Dissolve 15.0% (75.0 g.) gelatin and 0.8% (4.0 g.) Nährstoff Heyden in 500.0 cc. water.
(2) Dissolve 1.5% (7.5 g.) agar in 500.0 cc. water.
(3) Mix (1) and (2).

Sterilization: Not specified.

Use: Bacterial count of water.

Reference: Weiss (1920 p. 25).

1631. Deycke's Alkaline Albumin Gelatin Agar

Constituents:

1. Water	1000.0 cc.
2. Gelatin	50.0 g.
3. Agar	20.0 g.
4. Alkaline albumin	25.0 g.
5. NaCl	10.0 g.
6. Peptone	10.0 g.

Preparation:
(1) Add 2, 3, 4, 5 and 6 to (1).
(2) Heat slowly until the gelatin is dissolved. Stir.
(3) Neutralize the alkaline mixture by the careful addition of HCl, drop by drop, using litmus as an indicator.
(4) Add 2% of a soda solution containing two parts water to one part soda.
(5) Boil two hours in the steamer.
(6) Filter thru a thin layer of sterile cotton.
(7) Distribute into sterile petri dishes and solidify.

Sterilization: Sterilization given in the preparation.

Use: Diagnosis of cholera. Author reported that cholera colonies after 4 or 5 hours appeared similar to colonies 15 to 20 hours old in gelatin, having the same characteristics as the cholera colonies described by Koch, shiny with irregular edge. Liquefaction took place after 24 to 30 hours at 20–22°C.

Reference: Deycke (1895 p. 244).

1631a. Deycke's Albuminate Glycerol Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Alkali albuminate (from veal)	10.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Agar	20.0 g.
6. Glycerol	10.0 g.

Preparation:
(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Make alkaline by adding 33.0% soda.
(3) Filter.
(4) Tube.

Sterilization: Method not specified.

Use: Isolation of cholera vibrios. Author reported that only cholera, anthrax, diphtheria and tubercle bacilli grew.

Variants:
(a) The author used the following medium for the cultivation of diphtheria bacilli.
  (1) Dissolve 10.0 g. alkaline albuminate, 10.0 g. peptone, 5.0 g. NaCl, 20.0 g. agar-agar and 5.0 g. glycerol in 1000.0 cc. of distilled water.
  (2) Neutralize the alkaline mixture by the careful addition of HCl, drop by drop, using litmus paper as an indicator.
  (3) Make alkaline by adding 1.0% of a soda solution which contains two parts water and one part soda.
  (4) Soak at room temperature for one or more hours.
  (5) Boil in the steamer for ¾ to one hour.
  (6) Filter thru a layer of sterile cotton (may be filtered thru paper in a Unna steam funnel).
  (7) Distribute into sterile test tubes.
  (8) Sterilize once more in streaming steam for one-half hour.
  (9) Slant.
(b) Deycke and Voigtlander prepared a similar medium as follows:
  (1) Pour 300.0 cc. $Ba(OH)_2$ solution on finely chopped horse heart (or other fat free horse meat) and place in the incubator at 37°C. for 48 hours. This solution is usually complete at the end of this time.
  (2) Dilute (1) with 600.0 cc. of water.
  (3) Filter.
  (4) Heat to avoid the splitting of ammonia.
  (5) Neutralize the filtrate with HCl using litmus as an indicator.
  (6) To each 100.0 cc. of (5) add 400.0 cc. of water, 1.5 g. NaCl, 5.0 g. peptone, 25.0 g. glycerol and 10.0 g. agar. 1.5 to 2.0% glucose may be added.
  (7) Sterilization not specified.

References: Deycke (1894 p. 528), (1895 p. 242), Deycke and Voigtlander (1901 p. 621).

1632. Krumwiede and Pratt's Gelatin Serum Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone	20.0 g.
3. Agar	10.0 g.
4. Gelatin	80.0 g.
5. Serum, horse	500.0 cc.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Mix one part of 5 with two parts (1).
(3) Adjust to slightly alkaline to litmus.

Sterilization: Method not specified.

Use: Cultivation and preservation of stock cultures of fusiform bacilli.

Variants: The authors substituted ascitic fluid for horse serum.

Reference: Krumwiede and Pratt (1913 p. 200).

1633. Dunschmann's Bile Salt Peptone Agar (Bezançon)

Constituents:

1. Water	1000.0 cc.
2. Agar	30.0 to 40.0 g.
3. Gelatin	5.0 g.
4. Sodium taurocholate.	15.0 to 25.0 g.
5. Lactose	40.0 g.
6. Peptone (vegetable).	10.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1 (method not given).

Sterilization: Method not given.

Use: Enrichment of colon-typhoid group.

Reference: Bezançon (1920 p. 341).

1634. Capaldi's Peptone Gelatin Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone (Witte's)	20.0 g.
3. Gelatin	10.0 g.
4. Glucose	10.0 g.
5. NaCl	5.0 g.
6. KCl	5.0 g.
7. Agar (2.0%)	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Make alkaline by the addition of 10.0 cc. N/1 NaOH.

(3) Filter.

Sterilization: Method not given.

Use: Differentiation between colon and typhoid bacilli. The author reported that coli colonies were large milky colored by reflected and brown by transmitted light. Typhoid colonies were small, glistening and colorless. Abt used a similar medium to produce anthrax spores.

Variants:

(a) Abt used 2.0 g. Chapoteaut's peptone, 2.0 g. glucose, 20.0 g. gelatin and 15.0 g. agar instead of the amounts specified by Capaldi and omitted the KCl.

(b) Tanner used either 10.0 g. glucose or mannitol.

(c) Weiss made bacterial counts of water in a medium prepared as follows:

(1) Dissolve 15.0% gelatin and 1.0% peptone in 500.0 cc. of water.

(2) Add 0.15% Na_2CO_3 to (1).

(3) Dissolve 1.5% agar in 500.0 cc. of distilled water.

(4) Mix 500.0 cc. (2) with 500.0 cc. (3).

(5) Sterilization not specified.

References: Capaldi (1896 p. 475), Abt (1914 p. 151), Tanner (1919 p. 64), Weiss (1920 p. 25), Heinemann (1922 p. 35).

1635. Smyth's Egg Trypsinized Peptone Agar

Constituents:

1. Ringer's solution (see medium 180)	740.0 cc.
2. Egg albumin	250.0 cc.
3 Peptone	10.0 g.
4. Agar	7.5 g.

Preparation:

(1) Sterilize fresh eggs in bichloride of mercury solution followed by alcohol to remove the bichloride.

(2) Cut a small window in the side of an egg with a small sharp pointed scissors, in the same manner as one opens an egg to obtain the embryo.

(3) Remove the white with a sterile glass pipette (3.0 to 5.0 cc.) to a sterile Erlenmeyer flask, without breaking the yolk.

(4) Dissolve 10.0 g. of Witte's peptone in 200.0 cc. of Ringer's solution (See medium 180) at 80 to 90°C.

(5) Cool to 45°C. and add 0.5 cc. of trypsin powder suspended by shaking in 15.0 cc. of distilled water or Ringer's solution and digest for 3 hours at 40 to 45°C.

(6) Dilute (5) to one liter with Ringer's solution (see medium 180) and boil for 20 minutes and replace loss with distilled water.

(7) Filter and sterilize (The peptone solution so prepared may be kept for some time in a sterile flask).

(8) To (3) add an equal quantity of (7).

(9) Dissolve 15.0 g. agar in one liter Ringer's solution in the Arnold steam sterilizer or autoclave

(10) Clarify with egg.

(11) Distribute in small flasks.
(12) When ready for use, melt sterile (11) at 100°C., cool to 45°C. in a water bath and then add an equal volume of (8) (albumen peptone solution).
(13) Keep at 45°C. to prevent solidification before use.

Sterilization: Method of sterilization not given.

Use: To cultivate chick tissue in vitro.

Reference: Smyth (1914–15 p. 255).

1636. Czaplewski's Blood Nährstoff Heyden Agar (Klimmer)

Constituents:

1. Agar	1000.0 cc.
2. Nährstoff Heyden (1.0%)	10.0 g.
3. Blood, pigeon or dog.	

Preparation:
(1) Add 1.0% Nährstoff Heyden to neutral agar.
(2) Add several drops of pigeon or dog blood to (1).
(3) Solidify in a thin layer.

Sterilization: Not specified.

Use: Cultivation of influenza bacilli.

Reference: Klimmer (1923 p. 227).

1637. Norris et al. Blood Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone	20.0 g.
3. NaCl (5.0%)	50.0 g.
4. Glucose	20.0 g.
5. Agar	20.0 g.
6. Citrated blood (human)	100.0 cc.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Adjust slightly alkaline to phenolphthalein.
(3) Distribute in 20.0 cc. lots.
(4) Add 2.0 cc. human citrated blood per 20.0 cc. lot of medium.

Sterilization: Method not given.

Use: Cultivation of spirochetes.

Reference: Norris, Pappenheimer and Flournoy (1906 p. 281).

1638. Nicolle's Blood Peptone Agar (Behrens)

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	20.0 g.
3. Peptone	20.0 g.
4. NaCl	5.0 g.
5. Defibrinated blood, rabbit	2000.0 cc.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Distribute in test tubes in 1.0 cc. lots.
(3) Shortly before use the desired number of sterile agar tubes are melted in the water bath, cooled to 60°C. and two volumes of defibrinated rabbit's blood are added.
(4) Mix well and solidify in slanting position.

Sterilization: Sterilize by heating in the autoclave at 105° to 108°C. for 15 minutes.

Use: Cultivation of *Trypanosoma Brucei*.

Variants: Harvey prepared a similar medium by dissolving 0.5% peptone, 1.0% NaCl and 1.5% agar in water and mixing one part of this sterile melted agar cooled to 45°C. with one part defibrinated rabbit's blood also at 45°C.

References: Behrens (1914 p. 28), Harvey (1921–22 p. 74).

1639. Kelser's Blood Peptone Agar

Same as medium 724 but contains no glucose and contains 1.5% agar.

1640. Harvey's Blood Peptone Agar

Same as variant (b) 724, but solidified by the addition of 3.0% agar.

1641. Fildes' Blood Peptone Agar

Constituents:
1. Water.
2. Agar.
3. NaCl.
4. Peptone (Morson's).
5. Laked blood.
6. Serum.

Preparation:
(1) Collect ox or horse blood from a slaughter house.
(2) Defibrinate the blood by stirring with a sterilized large wooden stick wrapped in gauze.
(3) Lake the blood by the addition of an equal volume of distilled water.
(4) Distribute in 200.0 cc. lots into perfectly fitting glass stoppered bottles. (The author mentions that the medium should contain no suspended material, or it will not sterilize with the method employed. However, he does not specify any sedimentation of the mixture after the addition of the water.)

(5) Add chloroform to each bottle until 0.5% chloroform has been added.
(6) Add a drop of sterile oil to the stopper and fasten a dust cover tightly over the stopper.
(7) Place in the air incubator at 37°C. for 24 hours shaking constantly.
(8) After this time, remove a sample under aseptic conditions and test its sterility by mixing it with agar and incubating. (Time not specified.) If sterile, do not heat longer for heating tends to darken the medium.
(9) Collect horse or ox blood in tall sterile jars from the slaughter house.
(10) Allow to stand until all the corpuscles have deposited. It may require 14 days for this to take place. After five days examine the jars and pipette or siphon off the clear serum. Do not disturb the sediment.
(11) Distribute the clear serum in 200.0 cc. lots in perfectly fitting glass stoppered flasks.
(12) Add chloroform to each bottle until 0.5% chloroform has been added.
(13) Add a drop of sterile oil to the stopper and fasten a dust cover tightly over the stopper.
(14) Place the bottles in a water bath for one hour at 45°C. Shake occasionally.
(15) When the bottles have cooled, remove a sample under aseptic conditions and mix with agar. Incubate the mixture at 37° for five days to test sterility.
(16) Prepare a 2.5 to 3.0% agar solution in water containing salt and Morson's peptone only (meat infusion or derivatives not employed to decrease color).
(17) Add 1.0 cc. of (8) to 50.0 cc. of (15) under aseptic conditions, and add this mixture to 400.0 cc. of (16) and mix well.
(18) Tube thru a "hooded pipette."

Sterilization: Method of sterilization of agar not specified.

Use: Cultivation of meningococci and other pathogenic forms, pneumococci and streptococci. The author reported that this medium was very good for routine work with the meningococci. Pneumococci and streptococci developed abnormally large colonies on this medium.

Reference: Fildes (1917 p. 492).

1642. Golowkoff's Blood Peptone Agar (Uche)

Constituents:

1. Water	500.0 cc.
2. Agar (2.0%)	10.0 g.
3. Peptone	5.0 g.
4. NaCl	2.5 g.
5. Blood	500.0 cc.
6. Sugar (0.5%)	2.5 g.

Preparation:
(1) Prepare a 2.0% agar solution in water.
(2) Dissolve 3 and 4 in (1).
(3) Add an equal volume of blood (without any preparation) heated to 80°C., to (2).
(4) Boil for 15–20 minutes.
(5) While hot, separate the coagulum by pressing thru marly (a type of embroidery cloth).
(6) Filter.
(7) Add 0.5% sugar.

Sterilization: Not specified.

Use: Diagnosis of diphtheria.

Reference: Uche (1899 p. 393).

1643. Harvey's Blood Serum Peptone Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.
4. Lactose	10.0 g.
5. Neutral red (sat. soln.)	10.0 cc.
6. Agar	to solidify
7. Blood (defibrinated ox)	
8. Serum (ox)	

Preparation:
(1) Prepare a medium from 1, 2, 3 and 4 (see medium 607) and solidify with agar.
(2) Mix one part chloroform with 200.0 to 250.0 cc. of ox serum obtained under as nearly aseptic conditions as possible.
(3) Place in incubator 48 hours with occasional shaking.
(4) Test sterility.
(5) Mix equal parts distilled water and defibrinated ox blood obtained under as nearly aseptic conditions as possible.

(6) Mix one part chloroform with 200.0 to 250 parts (5).
(7) Place (5) in incubator 48 hours with occasional shaking.
(8) Test sterility.
(9) Add one part (8) to 20 parts (4) with sterile precautions, without shaking.
(10) Mix with sterile precautions at 45°C. one part (9) with seven parts sterile (1).
(11) Distribute with sterile precautions into test tubes.
(12) Slope.
(13) Stack the test tubes in the horizontal position.
(14) Test sterility by incubating 48 hours.

Sterilization: Method not given.

Use: Cultivation of meningococci. The author reported that the medium should be clear and free from all traces of red color.

Variants: Levinthal (Harvey) used one part (9) with 3 parts sterile agar.

Reference: Harvey (1921–22 p. 75).

1644. Harvey's Litmus Blood Peptone Agar

Constituents:

1. Distilled water	
2. Peptone	5.0 g.
3. NaCl	2.5 g.
4. Glucose	5.0 g.
5. Litmus solution	20.0 cc.
6. Defibrinated blood	

Preparation:
(1) Lake defibrinated blood with the smallest possible amount of sterile distilled water.
(2) Prepare a medium from 1, 2, 3, 4 and 5 as indicated in medium 595, and solidify with agar.
(3) Add a sufficient amount of (1) to (2) to give a deep color to the medium.

Sterilization: See medium 595 for sterilization of the agar.

Use: Cultivation of meningococci.

Reference: Harvey (1921–22 p. 76).

1645. Tochtermann's Serum Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	20.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Glucose	3.0 to 5.0 g.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Filter.
(3) Mix (2) with equal parts sheep blood serum or 3 parts serum to 2 parts agar.
(4) Filter.
(5) Tube.

Sterilization: Sterilize in the usual manner (method not given).

Use: Diagnosis of diphtheria. The author reported that diphtheria colonies after 24 hours were white.

Variants:
(a) Dieudonné gave the following method of preparation:
 (1) Allow sheep blood to stand for 24 hours.
 (2) Pour off the serum.
 (3) Dissolve 10.0 g. peptone, 5.0 g. NaCl and 20.0 g. agar in 1000.0 cc. water.
 (4) Filter and add 3.0 to 5.0 g. glucose to (3).
 (5) Add an equal volume (or 3 parts serum to 2 parts agar) of (2) and boil for 15 or 30 minutes.
 (6) Filter.
 (7) Distribute into test tubes and sterilize in streaming steam for 1 to 1½ hours on each of 3 successive days.
(b) Kolle and Wasserman prepared the medium as follows:
 (1) Dissolve 20.0 g. agar, 10.0 g. peptone, 5.0 g. NaCl, and 3.0 to 5.0 g. glucose in 1000.0 cc. water.
 (2) Mix (1) with equal parts sheep serum (or in the ratio of 2:3).
 (3) Boil for 75 to 90 minutes.
 (4) Filter.
 (5) Tube.
(c) Besson specified the use of Chapoteaut's or Witte's peptone, heated in the autoclave at 115 to 120°C. for 30 minutes and sterilized the medium at 115°C.
(d) Harvey specified the use of calf serum, boiled for 20 minutes and sterilized for 50 minutes at 100°C. or 30 minutes at 117°C.

References: Tochtermann (1895 p. 965), Kolle and Wassermann (1912 p. 406), Besson (1920 p. 53), Harvey (1921–22 p. 79), Klimmer (1923 p. 221).

1646. Browning's Telluric Acid Serum Agar (Wood)

Constituents:

1. Peptone water agar	1000.0 cc.
2. Serum, sheep	50.0 cc.
3. Telluric acid (1.0%)	9.0 cc.

Preparation: (1) Add 50.0 cc. of sterile sheep serum, and 9.0 cc. of a 1.0% solution of telluric acid in distilled water to 1000.0 cc. of peptone water agar.

Sterilization: Sterilize the serum by heating at 57°C. on several occasions. Method of sterilizing peptone water agar or telluric acid solution not given.

Use: Isolation of diphtheria bacilli.

Reference: Wood (1921 p. 562).

1647. Teague and Deibert's Serum Peptone Agar

Constituents:

1. Distilled water.
2. Peptone.
3. Agar.
4. NaCl.
5. Serum.
6. Red Blood Cells.

Preparation:

(1) Obtain sheep or rabbit serum from freshly drawn defibrinated blood.

(2) Heat (1) for 15 minutes at 55°C.

(3) Add 2.0 cc. of red blood cells (obtained by centrifuging defibrinated rabbit blood and removing the serum) to 10.0 cc. of physiological salt solution.

(4) Keep the temperature of (3) at 100°C. for 3 minutes.

(5) Shake (4) and allow to cool.

(6) Centrifuge (5) and obtain the supernatant fluid.

(7) Prepare a 5.0% peptone solution.

(8) Mix various amounts of (2), the supernatant fluid from (6) and (7) and add an agar solution containing 5.0 g. NaCl and 20.0 g. agar per 1000.0 cc.

Sterilization: Not specified.

Use: To study growth requirements of Unna-Ducrey bacillus. Author reported that excellent growth was obtained if red blood cell extract was added. Otherwise no growth.

Reference: Teague and Deibert (1922 p. 70).

1648. Emile-Weil's Pleuritic Serum Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone (Chapoteaut)	20.0 g.
3. Glucose	8.0 g.
4. Glycerol	20.0 g.
5. Agar	24.0 g.
6. Pleuritic serum (human)	250.0 cc.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Tube.

(3) To luke warm fluid (2) add one part human pleuritic serum to four parts agar.

Sterilization: Not specified.

Use: Cultivation of *Bacillus leprae.*

Variants: Emile- Weil gave the following variants:

(a) Used 40.0 g. glycerol, 4.0 g. glucose, 20.0 g. peptone, 20.0 g. agar, and mixed one part human pleuritic serum with two parts agar.

(b) Dissolved 10.0 g. NaCl, 8.0 g. glucose, 16.0 g. peptone and 16.0 g. agar in 1000.0 cc. water and mixed 2 parts of this solution with one part human pleuritic serum.

Reference: Emile-Weil (1905 p. 798).

1649. Bacto MacConkey's Agar (Dehydrated)

Constituents:

1. Water	
2. Bile, Ox, Bacto	5.0 g.
3. Lactose, Bacto	10.0 g.
4. Agar, Bacto	5.0 g.
5. Peptone, Bacto	20.0 g.
6. Neutral red	0.05 g.

Preparation:

(1) Dissolve 50.0 g. of Bacto MacConkey's Agar (Dehydrated) in 1000.0 cc. distilled water by boiling or autoclaving, preferably the latter.

(2) Restore the loss by the addition of water if necessary.

(3) If sterilized at 15 pounds for 20 minutes pH = 7.0±.

Sterilization: Sterilize in the autoclave at 15 pounds pressure for 20 minutes.

Use: General culture medium, and in water analysis.

Reference: Digestive Ferments Co. (1925 p. 12).

1650. Jordan's Basal Peptone Bile Agar

Constituents:

1. Bile (ox)	1000.0 cc.
2. Peptone (Witte)	10.0 g.
3. Agar	15.0 g.

Preparation:

(1) Dissolve 2, 3 and one of the added nutrients in 1000.0 cc. of undiluted fresh neutral ox bile. Avoid excessive heating.

(2) Filter without adjusting the reaction.

(3) Tube.

Sterilization: Sterilize in the autoclave for 3 minutes at 15 pounds pressure.

Use: To show inhibitory action of bile on *B. coli.* Author reported that both freshly isolated and strains of *B. coli* long under cultivation were inhibited.

Added nutrients: Jordan added 10.0 g. lactose.

Variants:

(a) Ecker specified the use of Difco peptone, used 10.0 g. lactose as an added nutrient and used the medium to study effect of bile on growth of *B. typhosus.* He reported that some strains were inhibited to a greater degree than others. Approximately the same results were obtained using 50.0% or 10.0% bile (diluted with distilled water).

(b) Ecker used a medium containing 10.0 g. glycerol instead of lactose as an added nutrient. The same results were reported as under variant (a) above.

(c) Ecker used the basal medium without any added nutrients. The same results were reported as under variant (a) above.

(d) Obst used the following medium to determine total count of *B. coli* in water analysis.

(1) Sterilize freshly collected ox bile and store at 1°C. until ready for use. Never use bile after one week following collection.

(2) Dissolve 10.0 g. lactose and 10.0 g. peptone in 1000.0 cc. of (1).

(3) Dissolve 15.0 g. of agar in (2).

(4) Filter. Great care must be taken in filtering so that medium be free from precipitate, or colonies cannot be counted.

(5) Sterilize (method not given).

References: Jordan (1912 p. 327), Ecker (1918 p. 97), Obst (1919 p. 76).

1651. Padlewsky's Bile Peptone Agar (Bezançon)

Constituents:

1. Water	1000.0 cc.
2. Peptone	20.0 g.
3. Bile	35.0 cc.
4. Lactose	10.0 g.
5. Malachite green (1.0%)	5.0 cc.
6. Agar	30.0 g.
7. Na_2SO_3 (10.0% soln.)	7.5 to 10.0 cc.

Preparation:

(1) Dissolve 20.0 g. peptone, 30.0 g. agar and 30.0 cc. beef bile in a liter of water (method not given).

(2) To 100.0 cc. of one add 0.5 cc. of a 1.0% aqueous solution of malachite green and 0.5 cc. of bile.

(3) Add 0.75 cc. to 1.0 cc. of a 10.0% Na_2SO_3 to each 100.0 cc. of medium.

Sterilization: Not specified.

Use: Cultivation of colon-typhoid group.

Reference: Bezançon (1920 p. 344).

1652. Northrup's Peptone Bile Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. Peptone	20.0 g.
4. NaCl	5.0 g.
5. Lactose	20.0 g.
6. Litmus (Standard solution Merck)	10.0 cc.
7. Bile (ox or sheep)	60.0 cc.

Preparation:

(1) Soak 15.0 g. of agar in 500.0 cc. of water over night.

(2) Dissolve 20.0 g. of peptone and 5.0 g. NaCl in 100.0 cc. hot water.

(3) After the agar has been digested add (2) to the sugar.

(4) Dissolve 20.0 g. lactose in 400.0 cc. hot water.
(5) Add (4) to (3).
(6) Add the contents of two ox sheep bile (about 60.0 cc.). The bile having been removed under aseptic conditions.
(7) Add 1.0% of a standard solution of Merck's purified litmus (Brown, C. W., Litmus media, 47th Ann. Rep., Michigan Board of Agriculture, pp. 127–129).
(8) Filter.

Sterilization: Not specified.

Use: Isolation of typhoid bacillus.

Variants: (a) Rector reported that colon colonies on the following medium were surrounded by a purplish red zone.

(1) Add 100.0 g. of dried ox bile and 10.0 g. Witte's peptone to 1000.0 cc. distilled water and boil.
(2) Filter.
(3) Add 1.5% shredded agar to (2) and heat for an hour in the Arnold sterilizer.
(4) Dissolve 10.0 g. glucose in a small quantity of hot water.
(5) Filter (3) thru absorbent cotton and cheese cloth.
(6) Prepare a 1.0% solution of neutral red.
(7) Add (4) and (6) to the filtrate of (5).
(8) Mix well.
(9) Tube in 6 to 10.0 cc. quantities.
(10) Autoclave for 10 minutes at 12 pounds pressure.

References: Northrup (1912 p. 420), Rector (1913 p. 154), Stitt (1923 p. 47).

1653. Bacto Whey Agar (Dehydrated)

Constituents:

1. Distilled water	1000.0 cc.
2. Whey (dry)	13.0 g.
3. Peptone (Bacto)	10.0 g.
4. NaCl	5.0 g.
5. Agar, Bacto	12.0 g.

Preparation:

(1) Dissolve 40.0 g. Bacto Whey Agar (Dehydrated) in 1000.0 cc. of distilled water by boiling or preferably autoclaving.
(2) If sterilized at 15 pounds pressure for 20 minutes pH = 6.5±.

Sterilization: Sterilize at 15 pounds pressure for 20 minutes.

Use: Culture medium, particularly for bacteria in dairy products.

Reference: Digestive Ferments Co. (1925 p. 14).

1654. Sabouraud and Noire's Whey Peptone Agar (Weil and Noire)

Constituents:

1. Water	500.0 cc.
2. Milk	1000.0 cc.
3. Peptone	10.0 g.
4. Sucrose	5.0 g.
5. Urea	1.75 to 2.0 g.
6. Agar	24.0 g.

Preparation:

(1) Precipitate the casein from one liter milk by the addition of 2.0 cc. of HCl.
(2) Filter thru a wet cloth.
(3) Dissolve 10.0 g. peptone, 5.0 g. sucrose, 1.75 to 2.0 g. urea in 500.0 cc. water.
(4) Add one-half volume of (3) to one volume of the filtrate from (2).
(5) Dissolve 1.6% agar in (4).
(6) Sterilize (method not given).
(7) Filter.
(8) Tube.

Sterilization: Sterilize again (method not given).

Use: Cultivation of gonococcus.

Variants: Bezançon prepared the medium as follows:

(1) Boil 1000.0 cc. of milk for 5 minutes.
(2) Add 2.0 cc. of HCl.
(3) Filter thru linen.
(4) Add one-half the volume of water to the whey.
(5) Neutralize by the addition of a 10.0% soda solution.
(6) Autoclave (5) for 10 minutes at 120°C.
(7) Filter.
(8) Dissolve 1.0% peptone, 1.0% sucrose or glucose, 0.3% urea and 1.6% agar in the filtrate by heating in the autoclave.
(9) Filter thru paper.
(10) Distribute in tubes.
(11) Sterilize for 10 minutes at 110°C.

References: Weil and Noire (1913 p. 1322), Bezançon (1920 p. 119).

1655. Tanner's Whey Peptone Agar

Constituents:

1. Whey	1000.0 cc.
2. Agar (1.5%)	15.0 g.
3. Peptone (Witte) (1.0%)	10.0 g.
4. Glucose (2.0%)	20.0 g.

Preparation:

(1) Add a few drops of acetic acid to boiling milk until the casein is precipitated.
(2) Filter.
(3) Neutralize or bring to +1.0%.
(4) Dissolve 1.0% peptone, 2.0% glucose and 1.5% agar in (2).
(5) Clarify with whites of eggs or dry albumin.
(6) Tube.

Sterilization: Method not given.

Use: Isolation of *B. vulgaricus*.

References: Heinemann and Hefferan (1909 p. 310), Tanner (1919 p. 71), Stitt (1923 p. 38).

1656. Committee A. P. H. A. Whey Peptone Agar

Constituents:

1. Whey	1000.0 cc.
2. Peptone (1.0%)	10.0 g.
3. Agar (1.5%)	15.0 g.

Preparation:

(1) Add sufficient rennet to a liter of skimmed milk at 41°C. to cause coagulation (1.0 cc. of liquid rennet in 20.0 cc. distilled water).
(2) When the curd is firm cut in fine pieces and steam for 40 minutes.
(3) Strain thru muslin.
(4) Adjust the reaction of the whey +1.5 and add 1.0% of dry peptone and 1.5% shredded agar.
(5) Steam for one hour.
(6) Readjust to an acidity of 1.5%.
(7) Cool to 60°C.
(8) Clarify with egg.
(9) Counterpose and boil over a free flame for 5 minutes.
(10) Filter thru cotton or a hot washed plaited filter paper.
(11) Tube.

Sterilization: Sterilize 15 minutes for 3 successive days in steam.

Use: General culture medium for milk analysis.

Variants:

(a) Fulmer and Grimes cultivated yeast found in cream and butter on a medium prepared as follows:
  (1) Dissolve 1.5% agar and 0.5% peptone (or omit peptone) in 1000.0 cc. of whey obtained from skim milk by coagulation with rennet.
  (2) Filter thru absorbent cotton.
  (3) Tube in 10.0 cc. portions.
  (4) Sterilize for 20 minutes at 15 pounds.
  (5) When used as a plate medium, add 1.0 cc. of 1.0% tartaric acid solution to each petri dish, to keep down bacterial growth. For use as solvents add 1.0 cc. of (4) to each tube after cooling to not above 45°C.

(b) Cunningham gave the following method of preparation:
  (1) Warm two liters of clean fresh skim milk in a large pot at 37°C.
  (2) Add sufficient rennet to curdle.
  (3) Allow to settle for 10 minutes.
  (4) Break the curd into large pieces by means of a stirring rod.
  (5) Heat to 80°C. to contract the clot and to express the whey.
  (6) Strain thru a cheese cloth.
  (7) Add 1.0% peptone and 0.5% NaCl.
  (8) Steam for 30 minutes.
  (9) Neutralize to tumeric paper.
  (10) Steam for one hour.
  (11) Dissolve 1.5% agar in (10).
  (12) Filter thru paper until clear.
  (13) Add 1.0% Andrades indicator.

References: Committee A. P. H. A. (1909 p. 287), Fulmer and Grimes (1923 p. 585), Klimmer (1923 p. 172), Cunningham (1924 p. 102).

1657. Huss' Whey Peptone Agar

Constituents:

1. Whey	1000.0 g.
2. Agar	20.0 g.
3. NaCl	5.0 g.
4. Peptone 1.0%	10.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1. (The peptone may be omitted.)

Sterilization: Method not given.

Use: Cultivation of aroma producing bacteria, *Bacillus esterificans*, Maassen and

Pseudomonas trifolii. Other investigators cultivated bacteria found in milk in similar media.

Variants:

- (a) Müller used 1.5% agar instead of 2.0%.
- (b) Meier mixed equal parts (whey from goat milk (see medium 2277)) and water and dissolved in the mixture 1.5% agar, 1.0% Witte's peptone (the peptone may be omitted) and 0.5% NaCl. The reaction was adjusted by the addition of KOH until turmeric paper was turned a weak brownish red.

References: Huss (1907 p. 58), Müller (1917 p. 390), Meier (1918 p. 435).

1658. Heller's Peptone Urine Agar

Constituents:

1. Urine	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.
4. Agar (1.5%)	15.0 g.

Preparation:

- (1) Secure human urine as near average specified gravity as possible.
- (2) Make weakly alkaline with soda. End point indicated by precipitation of salts.
- (3) Filter.
- (4) Add peptone, NaCl and 1.5% agar.
- (5) Boil.
- (6) Filter.
- (7) Tube.

Sterilization: Sterilize once in streaming steam.

Use: General culture medium.

Variants:

- (a) The author specified that any carbohydrate, alcohol, etc., might be added. The color and some inhibitory substances may be removed by animal charcoal.
- (b) Piorkowski used the following medium to differentiate between *Bacterium coli* and *Bacillus typhi abdomin.*
  - (1) Add 100.0 cc. urine and 0.5 g. peptone to a flask and plug with cotton. Urine should be fresh, clear, bright yellow, and of acid reaction. A urine of specific gravity of 1.012 gives the best medium.
  - (2) Steam for 15 minutes in a steamer.
  - (3) Dissolve 10.0 to 12.0% gelatin or agar in (2).
  - (4) Filter, using a hot water funnel or in a steamer.
  - (5) Distribute into 10.0 cc. lots and sterilize for 10 to 15 minutes on each of two days using the fractional method.
- (c) Piorkowski also gave the following medium for the differentiation of colon and typhoid organisms. He reported that the medium was blue. *Bact. coli communi* appeared as bluish-grey opaque damp colonies. Typhoid bacillus colonies delicate, transparent and appeared blue.
  - (1) Add 100.0 cc. of urine and 0.5 g. peptone to a flask and plug with cotton. A urine with a specific gravity of 1012 gave best results.
  - (2) Steam for 15 minutes in a steamer.
  - (3) Dissolve 2.0 g. of agar in (2).
  - (4) Filter, using a hot water funnel or filter in a steamer.
  - (5) Distribute in 10.0 cc. lots.
  - (6) To each tube add 8 drops of Böhmer's Hämatoxylyn solution (strength not given).
  - (7) Sterilize for 15 minutes on each of two successive days.
- (d) Matzuschita dissolved 2.0% agar, 1.0% peptone and 0.5% NaCl in urine.

References: Heller (1890 p. 893), Piorkowski (1896 pp. 687, 694), Matzuschita (1901–2 p. 214).

1659. Loeffler's Malachite Green Nutrose Peptone Agar (Harvey)

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone	20.0 g.
3. Lactose	50.0 g.
4. Glucose	10.0 g.
5. Nutrose	10.0 g.
6. Malachite green (2.0% chem. pure)	10.0 cc.
7. NaOH (N/1)	15.0 cc.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Cultivation of colon-typhoid group.

Variants: Klimmer gave the following method of preparation:

- (1) Prepare a 0.5% peptone solution.
- (2) Add 9.5 cc. of HCl to (1).

(3) Soak 3 in (2).
(4) Boil for 30 minutes.
(5) Add 7.5 cc. of normal KOH.
(6) Neutralize to litmus by the addition of soda solution.
(7) Add 5.0 cc. of a normal soda solution (143.0 g. crystalline soda per liter).
(8) Prepare a 10.0% nutrose solution.
(9) Add 100.0 cc. of (6) to (5).
(10) Flask.
(11) Sterilize on each of two successive days.
(12) Decant the clear agar from the sediment.
(13) Prepare a 2.0% solution of malachite green 120 (Höchst) in sterile distilled water (do not boil).
(15) Pour into Petri dishes.
(16) Leave the plates open until the agar has solidified.

References: Harvey (1921–22 p. 91), Klimmer (1923 p. 212).

1660. Raskin's Whey Peptone Agar

Constituents:

1. Whey	1000.0 cc.
2. Glycerol	50.0 cc.
3. Agar	5.0 to 7.0 g.
4. Peptone	10.0 g.

Preparation:

(1) Add 50.0 cc. of glycerol and 5.0 to 7.0 g. finely dried agar to 1000.0 cc. fresh milk.
(2) Allow to stand for 12 to 14 hours (at room temperature in the winter) and then boil for 75 to 90 minutes over a free flame. In order to prevent loss of water during the boiling place a lid over the container or steam for 3 to 3½ hours in a steamer. The coagulation of the casein takes place slowly.
(3) Press thru four folds of linen cloth separating the casein and liquid.
(4) The reaction of the fluid is slightly acid.
(5) Pour into a tall glass cylinder and place in a warm thermostat.
(6) After about 20 or 30 minutes two layers are formed, the lower layer being transparent and fat free, the upper layer yellowish white, containing the fat.
(7) Allow to cool, and remove the fat by means of a spoon.
(8) Heat the fat free portion to boiling and add 1.0% peptone.
(9) Add soda to neutralize the reaction.
(10) Filter until clear thru a paper in a hot water funnel.

Sterilization: Method not given.

Use: Cultivation of pathogenic organisms.

Variants:

(a) Raskin added 0.5% NaCl.

References: Raskin (1887 p. 358).

SUBGROUP II-C. SECTION 6

Basal or complete media containing agar and peptone (or other commercial digests) and extracts or infusions of animal origin.

A_1.* Not containing additional organic materials (exclusive of indicators).

B_1.* Infusions specified.

C_1. Indicators or dyes not added.

D_1. Meat infusion used.

Jacobi's Meat Infusion Salt Agar	1661
Zettnow's Meat Infusion Agar	1662
Besson's Phosphate Infusion Agar	1663
Smith's Infusion Agar	1664
Francis' Basal Infusion Agar	1665
Deycke's Alkali Albuminate Agar	1666
Harvey's Basal Infusion Agar	1667
Krasnow's et al. Meat Infusion Agar	1668

D_2. Infusions of other tissues or special organs, etc. used.

Dopter's Liver Infusion Agar (White)	1669
Richardson's Mucose Infusion Agar	1670
Lichtenstein's Blood Clot Infusion Agar	1671
Stuart's Stomach-Liver Infusion Agar	1672
Bailey's Hormone Agar	1673
Fasiani & Zironi's Veal Autolysate Peptone Agar	1674

C_2. Indicators or dyes added.

Harvey's Basal Indicator Infusion Agar	1675
Jordan and Victorson's Lead Acetate Infusion Agar	1676
Elser and Huntoon's Basal Litmus Infusion Agar	1677
Emile-Weil's Neutral Red Infusion Agar	1678
Lentz and Tietz's Malachite Green Infusion Agar	1679
Zielleczky's Phenolphthalein Infusion Agar	1680
Worbitzki's China Green Infusion Agar	1681

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* See page 482 for A_2 and B_2.

- Teague's Victoria Blue Infusion Agar........ 1682
- Drennan and Teague's Crystal Violet Infusion Agar........ 1683
- Meyer & Batchelder's Sulphite Gentian Violet Infusion Agar........ 1684
- Muller's Indicator Infusion Agar........ 1685
- Friedberger & Joachimoglu's Tellurite Placenta Infusion Agar........ 1687
- Ragit Agar........ 1688

B_2.* Extracts specified.

C_1. Indicators or dyes not added.

D_1. Complete dehydrated media commercially prepared.

- Bacto Nutrient Agar (Dehydrated)........ 1689
- Bacto Nutrient Phosphate Agar (Dehydrated)........ 1690
- Bacto Nutrient Agar 1.5% (Dehydrated)........ 1691
- Bacto Nitrate Agar (Dehydrated)........ 1692

D_2. Media prepared from its constituents.

- Bannings' Basal Meat Extract Agar........ 1693
- Heim's Meat Extract Salt Agar........ 1694
- Heinemann's Meat Extract Agar........ 1695
- Glaessner's Nährstoff Heyden Extract Agar........ 1696
- Guth's Selenicate Extract Agar........ 1697
- Conn and Breed's Nitrate Extract Agar........ 1698
- Kotlar's Pancreas Peptone Agar........ 1699

C_2. Indicators or dyes added.

- Bacto Andrade Maltose Agar (Dehydrated)........ 1700
- Bacto Andrade Mannite Agar (Dehydrated)........ 1701
- Bacto Andrade Dextrose Agar (Dehydrated)........ 1702
- Bacto Andrade Lactose Agar (Dehydrated)........ 1703
- Bacto Andrade Saccharose Agar (Dehydrated)........ 1704
- Percival's Basal Litmus Extract Agar........ 1705
- Committee S. A. B. Lead Acetate Extract Agar........ 1706
- Conradi's Brilliant Green Picric Acid Extract Agar (Bezançon)........ 1707
- Klinger's Malachite Green Extract Agar........ 1708

B_3. Whether infusions or extracts employed not definitely specified.

C_1. Indicators or dyes not added.

- Köhler's Basal Agar........ 1709
- Wurtz's Nutrient Agar........ 1710
- Warden's Salt Agar........ 1711
- Strószner's Regenerated Agar........ 1712

C_2. Indicators or dyes added.

- Besson's Basal Litmus Agar........ 1713
- Mandelbaum's Basal Rosolic Acid Agar........ 1714
- Zipfel's Regenerated Drigalski's Agar 1715
- Rothberger's Indicator Agar........ 1716
- Omelianski's Indicator Agar........ 1717
- Burnet and Weissenbach's Lead Acetate Agar........ 1718
- Noeggerath's Indicator Agar (Besson)........ 1719
- Gasser's Fuchsin Agar........ 1720
- Krumwiede and Pratt's Dahlia Agar 1721

A_2. Containing additional organic materials.

B_1.* All additional material of known chemical composition.

C_1.† Infusions specified.

D_1.† Only one additional source of carbon added.

E_1. Carbohydrates employed.

F_1. Monosaccharides, only, used.

G_1. Indicators or dyes not added.

- Meier's Glucose Infusion Agar........ 1722
- Hitchens' Glucose Infusion Agar........ 1723
- Jackson and Muer's Liver Infusion Agar........ 1724
- Hall's Testicular Infusion Agar........ 1725

G_2. Indicators or dyes added.

- Kligler's Lead Acetate Infusion Agar........ 1726
- Gage and Phelps' Neutral Red Infusion Agar........ 1727
- Harvey's Malachite Green Infusion Agar........ 1728

F_2. Disaccharides, only, used.

- Endo's Fuchsin Sulphite Infusion Agar........ 1729
- Wurtz' Litmus Lactose Agar........ 1730
- Bitter's China Blue Malachite Green Agar........ 1731

F_3. Polysaccharides used.

- Guth's Alizarine Lactose Agar (Klimmer)........ 1732
- Harvey's Starch Agar........ 1733
- Gassner's Metachrone Yellow Water Blue Infusion Agar........ 1734
- Torrey's Brom Cresol Purple Lactose Agar........ 1735

E_2.‡ Alcohols employed.

- Abbott's Glycerol Infusion Agar........ 1736

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* See B_3.

* See page 484 for B_2.

† See page 483 for C_2 and D_2.

‡ See E_3, page 483.

Kowalski's Glycerol Lung Infusion Agar (Dittrich) 1737
Henssen's Glycerol Kidney Agar ... 1738
Harvey's Mannitol Infusion Agar ... 1739
E_3. Organic acids or their salts employed.
Omelianski's Formate Agar 1740
Harvey's Salicylate Infusion Agar .. 1741
D_2. More than one additional source of carbon added.
E_1. Two or more carbohydrates employed.
de Gasperi and Savini's Glucose Lactose Agar 1742
Teague and Clurman's Eosin Brilliant Green Agar 1743
Kan-Ichiro, Morishima's Lead Acetate China Blue Agar 1744
Aronson's Fuchsin Sulphite Agar (Harvey) 1745
E_2. Carbohydrates and alcohols employed.
v. Szaboky's Glycerol Lung Agar ... 1746
Hulton-Frankel's Inositol Infusion Agar 1747
Robinson and Rettger's Opsine Infusion Agar 1748
E_3. Carbohydrates with an organic nitrogenous compound of known chemical composition.
Lubenau's Lactose Caffeine Agar .. 1749
Harvey's Caffeine Endo Agar 1750
Gäthgen's Caffeine Fuchsin Sulphite Agar (Bezançon) 1751
C_2.* Extracts specified.
D_1. Only one additional source of carbon added.
E_1. Carbohydrates employed.
F_1. Monosaccharides, only, added.
Viehoever's Basal Glucose Extract Agar 1752
Bacto Dextrose Agar (Dehydrated). 1753
Henneberg's Glucose Extract Agar .. 1754
Oldekop's Neutral Red Glucose Extract Agar 1755
F_2. Disaccharides, only, added.
Bacto Purple Lactose Agar (Dehydrated) 1756
Bacto Litmus Lactose Agar (Dehydrated) 1757
A. P. H. A. Litmus Lactose Extract Agar (1917) 1758
Endo's Fuchsin Sulphite Agar (Heinemann) 1759
Hirschbruch & Schwer's Crystal Violet Litmus Lactose Agar 1760
Chesney's Indicator Lactose Agar .. 1761
Salomonsen's Sucrose Extract Agar (Besson) 1762
F_3. Polysaccharides, only, added.
Hesse's Starch Extract Agar (Stokes and Hachtel) 1763
Committee S. A. B. Starch Extract Agar 1764
E_2. Alcohols, acids or their salts employed.
Coplin and Bevan's Glycerol Extract Agar 1765
E_3. Other organic materials employed.
Piettre and de Souza's Citric Acid Extract Agar 1766
Fawcus' Dye Bile Salt Agar (Bezançon) 1767
Percival's Urea Extract Agar 1768
D_2. More than one additional source of carbon added.
E_1. Organic nitrogen added.
Gaehtgen's Caffeine Endo Agar 1769
Wilson and Darling's Brilliant Green Bile Salt Agar 1770
Olszewski and Köhler's Endo Bile Salt Agar 1771
E_2. Organic nitrogen not added.
F_1. Containing carbohydrates and alcohols.
Bacto Saccharose-Mannitol Agar (Dehydrated) 1772
Hesse's Lactose Glycerol Agar (Stokes and Hachtel) 1773
Schnürer's Saponin Glycerol Agar .. 1774
F_2. Containing carbohydrates, without alcohols.
G_1. Containing two carbohydrates.
Bacto Russell Double Sugar Agar (Dehydrated) 1775
Nichols and Woods' Russell Double Sugar Agar 1776
Bailey and Lacey's Phenol Red Lead Acetate Agar 1777
Holt-Harris and Teague's Eosine Methylene Blue Agar 1778
Krumwiede, Pratt and McWilliams' Brilliant Green Agar 1778a
Aronson's Fuchsin Sulphite Agar ... 1779
Hesse's Malachite Green Agar (Klimmer) 1780
G_2. Containing more than two carbohydrates.
Bacto Krumwiede Triple Sugar Agar (Dehydrated) 1781
Krumwiede and Kohn's Triple Sugar Agar 1782
Amoss' Four Sugar Agar 1783

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* See C_3, page 484.

Amoss' Sucrose Salicin Agar (Topley and Ayrton ... 1784

C_3. Whether infusions or extracts employed not definitely specified.

D_1. Only one additional source of carbon added.

E_1. Carbohydrates employed.

F_1. Monosaccharides only added.

Frost's Glucose Agar ... 1785
Mankowski's Indigo Carmine Glucose Agar ... 1786
Rivas' Glucose Agar ... 1787
Hall and Elliefson's Gentian Violet Glucose Agar ... 1788
Wilson and Blair's Sulphite Glucose Agar ... 1789
Scheffler's Neutral Red Glucose Agar ... 1790
Martin and Loiseau's Glucose Litmus Agar ... 1791

F_2. Dissaccharides, only, added.

Frost's Lactose Agar ... 1792
Wurtz's Litmus Lactose Agar ... 1793
Gassner's Metachrome Yellow Water Blue Lactose Agar (Klimmer) ... 1794
Hirschbruch and Schwer's Azolitmin Crystal Violet Lactose Agar .. 1795
Ramond's Rubine Acid Lactose Agar 1796
Delta's Fuchsin Lactose Agar ... 1797
Kindberg's Fuchsin Malachite Green Agar ... 1798
Bitters' China Blue Malachite-green Agar (Klimmer) ... 1799
Liebermann and Acels' Congo Red Agar (Klimmer) ... 1800
Massini's Triple Dye Lactose Agar. 1801

F_3. Polysaccharides, only, added.

Lange's Starch Agar ... 1802
Hoffman's Nitrate Starch Agar ... 1803
Hoffman's Nitrite Starch Agar ... 1804
Khouvine's Cellulose Agar ... 1805
Scales' Salt Cellulose Agar ... 1806

E_2. Alcohols employed.

Cantani's Basal Glycerol Agar ... 1807
Wurtz's Glycerol Agar ... 1808
Scheffler's Indicator Glycerol Agar. 1809
Mandelbaum's Rosolic Acid Glycerol Agar ... 1810
Heinemann's Litmus Mannitol Agar. 1811

E_3. Organic materials other than carbohydrates or alcohols employed.

Penfold's Phenylacetate Agar ... 1812
Wurtz's Phenol Agar (Copeland) ... 1813
Penfold's Monochlorhydrin Agar ... 1814
Jacobson's Ethylcinnamic Ether Agar ... 1815
Hurler's Caffeine Agar ... 1816
Finger, Ghon and Schlagenhaufer's Urea Agar ... 1817

D_2. More than one additional source of carbon added.

E_1. Containing two or more carbohydrates.

Russell's Double Sugar Agar ... 1818

E_2. Containing carbohydrates and alcohols.

Thoinot and Masselin's Glucose Glycerol Agar ... 1819
Thoinot and Masselin's Sucrose Glycerol Agar ... 1820
Kligler and Defandorfer's Double Sugar Agar ... 1821
Kendall and Ryan's Sucrose Mannitol Agar ... 1822
Hulton-Frankel and MacDonald's Inositol Dextrin Agar ... 1823

E_3. Containing carbohydrates and organic acids or their salts.

Kitasato's Glucose Formate Agar (Tanner) ... 1824
Söhngen and Fol's Glucose Butyrate Agar ... 1825
MacDonald's Glucose Lactic Acid Agar ... 1826

E_4. Containing carbohydrates and organic nitrogenous compounds.

Müller's Lactose Tartrate Agar ... 1827
Kligler's Lead Acetate Glucose Agar. 1828
MacConkey's Lactose Bile Salt Agar (Heinemann) ... 1829

E_5. Containing alcohols and organic acids or their salts.

Fleming's Oleic Acid Glycerol Agar. 1830

B_2. Containing material of unknown chemical composition.

C_1.* Extracts specified.

D_1. Containing animal tissue or cells, or their derivatives.

Rosenow and Towne's Ascitic Fluid Kidney Agar ... 1831
Rosenow's Glucose Brain Agar (Haden) ... 1832
Goldberger's Glucose Alkaline Egg Agar (Abbott) ... 1833
Goldberg's Meat Infusion Extract Agar (Stitt) ... 1834
Dimitroff's Egg Agar ... 1835

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* See page 485 for C_2.

Dunschmann's Bile Salt Gelatin Agar 1836

D_2. Containing animal fluids.

E_1. Ascitic fluid specified.

Paneth's Glucose Ascitic Fluid Agar. 1837

E_2. Blood specified.

Baehr and Plotz's Blood Ascitic Fluid Agar 1838
Reed and Orr's Blood Agar 1839
Becker's Defibrinated Blood Agar. 1840
Esch's Alkaline Hemoglobin Ragit Agar 1841

E_3. Bile specified.

Padlewsky's Malachite Green Bile Agar 1842

E_4. Variety of body fluids specified.

Fildes' Body Fluid Agar 1843
Haner and Frost's Milk Body Fluid Agar 1844
Bacto Conradi-Drigalski Agar (Dehydrated) 1845

D_3. Containing animal secretions or excretions or their derivatives.

Haner and Frost's Milk Agar 1846
Eldredge and Roger's Whey Agar ... 1847
Ayers, Courtland and Mudge's Milk Powder Agar 1848
Conradi-Drigalski's Crystal Violet Litmus Agar (Park, Williams and Krumwiede) 1849
Tausz and Peter's Ragit Nutrose Agar 1850
Hunter's Trypsinized Casein Extract Agar 1851
Zoller's Citrate Milk Agar 1852

C_2.* Infusions specified.

D_1.† Containing unknown constituents of animal origin.

E_1. Containing animal tissues or cells or their derivatives.

F_1. Tissue employed.

Smillie's Tissue Infusion Agar 1853
van Riemsdijk's Liver Infusion Agar. 1854
Williams' Tissue Infusion Agar 1855
Olitsky and Gates' Ascitic Fluid Kidney Agar 1856
Pelouze & Viteri's Brain Veal Infusion Agar 1857

F_2.‡ Blood Cells employed.

Avery's Oleate Blood Cell Agar (Harvey) 1858
Brown and Orcutt's Blood Cell Agar. 1859
Behren's Blood Cell Agar 1860

F_3. Egg or other materials employed.

Sacquépée and Delater's Egg Albumin Infusion Agar 1861
Emile-Weil Egg Yolk Infusion Agar. 1862
Huntoon's Hormone Agar 1863
Harvey's Alkaline Egg Agar 1864
Meyers' Basal Mollusk Infusion Agar 1865
Faroy and Chavaillon's Egg Albumin Serum Agar 1866
Huntoon's Hormone Blood Agar ... 1867
Torrey and Buckell's Ascitic Fluid Egg Agar 1868
Williams-Burdick's Modified Petroff's Egg Infusion Agar (Roddy). 1869
Chapin's Egg Yolk Urine Agar 1870
Harvey's Sucrose Egg Agar 1871
Leboeuf's Egg White Liver Infusion Agar 1872

F_4. Tissue derivatives employed.

Besson's Gelatin Infusion Agar 1873
Frothingham's Gelatin Infusion Agar 1874
Krause's Gelatin Urea Agar 1875
MacNeal and Kerr's Gelatin Agar .. 1876
Supplee's Nutrose Gelatin Agar (Ayers and Johnson) 1877
Kinsella, Brown and Garcia's Nutrose Gelatin Agar 1878
Ayers and Johnson's Casein Gelatin Agar 1879
Frazier's Gelatin Agar 1880

E_2. Containing animal fluids.

F_1.* Blood or its derivatives employed.

G_1.* Defibrinated blood specified.

H_1. Additional organic constituents (if any) of known chemical composition.

Duval and Lewis' Glucose Blood Agar 1881
Torrey's Glucose Blood Agar 1882
Erickson and Albert's Testicular Blood Agar 1883
Harvey's Lactose Blood Agar 1884
Harvey's Saponin Blood Agar 1885
Warden's Blood Veal Agar 1886
Wherry and Ervin's Glycerol Blood Agar 1887
Elser and Huntoon's Basal Blood Infusion Agar 1888
North's Gelatin Blood Agar (Kligler) 1889
Dieudonne's Alkaline Blood Agar (Harvey) 1890

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* See C_3, page 487.

† See page 486 for D_2.

‡ See F_3 and F_4.

* See page 485 for F_2 and G_2.

- Fildes' Blood Digest Agar (Kristensen) 1891
- MacNeal's Blood Infusion Agar 1892
- Smedley's Blood Infusion Agar ... 1893
- Harvey's Peptic Blood Digest Agar. 1894
- Bailey's Hormone Blood Agar 1895

H_2. One or more of additional organic constituents of unknown chemical composition.

- Harvey's Ascitic Fluid Blood Agar .. 1896

G_2. Whole blood specified.

H_1. Blood citrated or oxalated.

- Harvey's Oxalated Blood Agar 1897
- Wolbach, Chapman and Stevens' Citrated Blood Agar 1898
- Noguchi's Serum Plasma Agar 1899
- Soparkar's Citrated Blood Agar (Liston) 1900
- Hirsch and McKinney's Chocolate Agar 1901
- Harvey's Glucose Blood Agar 1902

H_2. Blood neither citrated nor oxalated.

- Grassberger's Blood Agar 1903
- Ruediger's Blood Agar 1904
- Dieudonne's Alkaline Blood Agar (Tanner) 1905
- Sherwood and Downs' Glucose Blood Agar 1906
- Hall's Testicular Infusion Blood Agar (Stitt) 1907
- Cutler's Blood Clot Infusion Agar (Klimmer) 1908
- Harvey's Trypsinized Blood Agar .. 1909
- Harvey's Hydrolyzed Blood Agar .. 1910

G_3. Hemoglobin specified.

- Kristensen's Hemoglobin Infusion Agar 1911
- Brown and Orcutt's Hemoglobin Infusion Agar 1912
- Esch's Alkaline Hemoglobin Infusion Agar (Tanner) 1913

F_2. Serum employed.

- Sherwood and Downs' Basal Serum Agar 1914
- Harvey's Placenta Blood Serum Agar 1915
- Veillon's Serum Agar 1916
- Kutscher's Serum Placenta Agar .. 1917
- Bezançon's Serum Placenta Agar ... 1918
- Shamine's Nucleic Acid Serum Agar. 1919
- Robey, et al., Glucose Serum Agar .. 1920
- Noguchi's Ringer Solution Serum Agar 1921
- Todd's Lactose Serum Agar 1922
- Harvey's Telluric Acid Serum Agar. 1923
- Francis' Cystine Serum Agar (Stitt). 1924

F_3. Ascitic fluid employed.

- Elser and Huntoon's Basal Ascitic Fluid Agar 1925
- Gilbert and Humphrey's Tellurite Serum Agar 1926
- Scholtz's Ascitic Fluid Agar 1927
- Kiefer's Ascitic Fluid Agar (Abel) .. 1928
- Torrey and Buckell's Urine Ascitic Fluid Agar 1929
- Watabiki's Whey Ascitic Fluid Agar. 1930
- Esch's Maltose Ascitic Fluid Agar .. 1931

F_4. Animal fluids other than blood, serum, or ascitic fluid employed.

- Steinschneider's Hydrocele Fluid Agar 1932
- Heiman's Pleuritic Serum Agar 1933
- Lentz and Tietz's Malachite Green Bile Agar (Klimmer) 1934

E_3. Containing animal secretions, excretions or their derivatives.

F_1. Milk or its derivatives employed.

G_1. Nutrose used.

- Lentz and Tietz's Malachite Green Nutrose Bile Agar (Klimmer) ... 1935
- V. Drigalski and Conradi's Crystal Violet Nutrose Agar 1936
- Galli-Valerio's Neutral Red Nutrose Agar 1937
- Teague and Travis' Eosin Bismark Brown Nutrose Agar 1938
- Gassner's Nutrose Agar 1939
- Loeffler's Malachite Green Nutrose Agar (Roddy) 1940
- Schmitz's Nutrose Blood Clot Infusion Agar 1941

G_2. Nutrose not used.

- Harvey's Milk Agar 1942
- Lubenau's Caffeine Whey Agar 1943
- Harvey's Whey Infusion Agar 1944

F_2. Milk or its derivatives not employed.

- Schloffer's Urine Infusion Agar 1945
- Ficker's Glycerol Sputum Agar ... 1946
- Szaboky's Sputum Lung Infusion Agar 1947
- Costa and Boyer's Gum Infusion Agar 1948
- Tulloch's Ox Heart Infusion Pea Flower Agar 1949a

D_2. Not containing additional materials of unknown chemical composition of animal origin.

C_3. Whether infusions or extracts were employed not definitely specified.
D_1. Containing tissues, cells or their derivatives.
E_1. Egg employed.
F_1. Yolks only used.
- Capaldi's Egg Yolk Agar........... 1949
- Nastiukoff's Egg Yolk Nutrient Agar (Rechtsamer)............... 1950
- Bezançon and Griffon's Glycerol Egg Yolk Agar.................. 1951
- Pergola's Tellurite Egg Yolk Agar.. 1952

F_2. Whites of eggs only used.
- Lipschutz's Egg Albumin Agar.... 1953
- Krumwiede, Pratt and Grund's Egg Albumin Agar................... 1954
- Oberstadt's Egg Albumin Agar..... 1955

F_3. Whole egg (white and yolk) used.
- Besredka and Jupille's Egg Agar (Besson)......................... 1956
- Scales' Whole Egg Agar............ 1957
- Stitt's Glycerol Egg Agar.......... 1958
- Robertson's Alkaline Egg Agar (Park, Williams and Krumwiede). 1959

E_2. Tissues or their derivatives other than eggs employed.
F_1. Tissue used.
- Cantani'juns' Sperm Agar.......... 1960
- Pettersson's Brain Ascitic Fluid Agar........................... 1961
- Smith and Taylor's Fetus Agar.... 1962
- Noguchi's Ascitic Fluid Tissue Agar. 1963
- Gozony's Kidney Agar............. 1964
- Duval's Trypsinized Tissue Agar... 1965

F_2. Tissue derivatives used.
G_1. Containing gelatin.
- Thoinot and Masselin's Gelatin Agar. 1966
- Fremlin's Phosphate Gelatin Agar.. 1967

G_2. Not containing gelatin.
- Vierling's Fat Agar................ 1968
- Abe's Meat Water Infusion Agar... 1969
- Esch's Hydrolyzed Meat Agar (Köhlisch and Otto).............. 1970
- Wellman's Placenta Infusion Agar.. 1971

D_2.* Containing animal fluids.
E_1. Blood or its derivatives employed.
F_1. Defibrinated blood used.
G_1. Additional materials, if any, inorganic, (exclusive of dyes).
- Orcutt and Howe's Fat Blood Agar. 1972
- Dieudonne's Alkaline Blood Agar.. 1973
- Pilon's Alkaline Blood Agar....... 1974
- Fildes' Pepsinized Blood Agar...... 1975
- Carpano's Hemolyzed Blood Agar.. 1976

G_2. At least one additional material organic.
- Mandelbaum's Lactose Blood Agar. 1977
- Thompson's Glucose Plasma Agar.. 1978
- Avery's Oleate Blood Agar (Stitt). 1979
- Esch's Ascitic Fluid Blood Agar.... 1980
- Liston's Trypsinized Casein Blood Agar........................... 1981

F_2.* Whole blood used.
G_1. Blood citrated or oxalated.
- Bernstein's Basal Blood Agar...... 1982
- Wordley's Oxalated Blood Agar.... 1983
- Wilson and Darling's Laked Blood Agar........................... 1984
- Wilson and Darling's Lactose Blood Agar........................... 1985
- Stitt's Glycerol Blood Agar (Chocolate Agar)...................... 1986
- Besson's Citrated Blood Agar...... 1987

G_2. Blood neither citrated nor oxalated.
H_1. Additional constituents, if any, inorganic, (exclusive of dyes).
- Bezançon, Griffon and LeSourd's Blood Agar...................... 1988
- Fleming's Brilliant Green Blood Agar........................... 1989
- Hachla and Holobut's Alkaline Blood Agar...................... 1990
- Matthews' Trypsinized Blood Agar. 1991

H_2. At least one of the added constituents organic.
- Bieling's Glucose Blood Agar...... 1992
- Mandelbaum and Heinemann's Glycerol Blood Agar (Kolle and Wassermann).................... 1993
- Wassermann's Nutrose Blood Agar (Abel).......................... 1994
- Savini and Savini-Castano's Bacteria Blood Agar................. 1995
- Bieling's Optochin Hydrochloride Blood Agar..................... 1996

F_3. Hemoglobin used.
- Heim's Hemoglobin Agar.......... 1997
- Esch's Alkaline Hemoglobin Agar.. 1998
- Kabeshima's Alkaline Hemoglobin Agar........................... 1999
- Besson's Glycerol Hemoglobin Agar (Tanner)........................ 2000
- Kabeshima's Hemoglobin Extract Agar........................... 2001

E_2 † Serum employed.
- Finger, Ghon and Schlagenhaufer's Dialyzed Serum Agar............ 2002
- Müller's Serum Agar............... 2003

See D_3 and D_4, page 488.

* See F_3.

† See E_3 and E_4, page 488.

Joas' Alkaline Serum Agar (Klimmer)............................ 2004
Muhlen and Hoffman's Glucose Serum Agar (Stitt)............... 2005
Cantani's Glycerol Serum Agar (Besson)......................... 2006
Kodama's Fuchsin Sulphite Serum Agar............................ 2007
Wassermann's Nutrose Serum Agar. 2008
Kliglers' Nasal Secretion Serum Agar........................... 2009
Meyers' Tuberculin Agar........... 2010
Douglas' Tellurite Trypsinized Serum Agar........................ 2011
Czaplewski's Alkaline Serum Glucose Agar....................... 2012
Klein's Alkaline Serum Agar....... 2013
Frost, Charlton and Little's Milk Serum Agar...................... 2014

E_3. Ascitic fluid employed.
Salomon's Basal Ascitic Fluid Agar. 2015
Rosenow's Glucose Ascitic Fluid Agar............................ 2016
Ruediger's Inulin Ascitic Fluid Agar. 2017
Klimenko's Glycerol Ascitic Fluid Agar............................ 2018
Veilion's Ascitic Fluid Agar........ 2019
Herrold's Phosphate Ascitic Fluid Agar............................ 2020

E_4. Bile employed.
Loeffler's Dye Bile Agar (Abel).... 2021

D_3. Containing animal secretions or excretions.

E_1. Milk or its derivatives employed.
Hasting's Milk Agar............... 2022
Valleti's Whey Agar................ 2023
Klimmer's Casein Agar............. 2024

E_2. Milk or its derivatives not employed.
Mayer's Mucin Agar............... 2025
Fichtner's Sputum Agar............ 2026
Finger, Ghon and Schlagenhaufer's Urine Agar....................... 2027

D_4. Containing materials of plant origin.
Eberson's Yeast Infusion Agar..... 2028

1661. Jacobi's Meat Infusion Salt Agar

Constituents:

1. Meat infusion..............	1500.0 cc.
2. Peptone (siccum)...........	15.0 g.
3. NaCl......................	7.5 g.
4. Agar-agar..................	22.5 g.

Preparation:

(1) Method of preparation of meat infusion not given.
(2) Add 2, 3 and 4 to (1).
(3) Heat over a free flame until the materials are completely dissolved.
(4) Make up to the original volume by the addition of water.
(5) Add Na_2CO_3 or sodium phosphate until the reaction is slightly alkaline.
(6) Pour into a flask and steam until the albuminous material still to be removed is completely separated (usually 2 hours if sodium phosphate is used and longer if Na_2CO_3).
(7) Filter thru cotton, using compressed air to effect a fast filtration.
(8) Distribute into smaller flasks.
(9) Heat in streaming steam for 2 hours (if the glassware has been sterilized in the autoclave).
(10) Finally distribute into sterile test tubes that have been autoclaved in the autoclave for 2½ hours. (The tubes are contained in an enamel container in the autoclave. The inner temperature reaching about 150°C.).

Sterilization: Not specified.

Use: General culture medium.

Variants: The following authors prepared similar media as indicated:

(a) Schultz (1891).
  (1) Place 500.0 g. of the best quality meat, without fat or tendons, in a glass container fitted with a lid.
  (2) Pour 1300.0 cc. of distilled water over the meat.
  (3) Store in a cool place until the next day.
  (4) Filter thru 4 thicknesses of cloth and press the remaining meat to obtain as much fluid as possible.
  (5) Pour the filtrate into a kettle, add 10.0 g. peptone (siccum), 5.0 g. NaCl and the whites of two eggs, beaten up in two or three volumes of water.
  (6) Boil under a gas flame for 15 minutes.
  (7) Adjust to faint alkalinity, using phenolphthalein as an indicator, and the end point being a faint red color.
  (8) Pour into an iron kettle, add 100.0 cc. distilled water; boil strongly for 5 minutes and filter.
  (9) Add 15.0 g. agar-agar to (8).

(10) Place in a steamer for 12–15 hours.
(11) Filter thru a hot funnel.
(12) The steaming for 12–15 hours is sufficient for sterilization.

(b) Schultz (1891).
(1) Place 3 to 4 liters of distilled water in an enamel container and add 20.0 g. finely cut up agar-agar to it.
(2) Boil two hours in a closed vessel.
(3) Place 500.0 g. of the best quality meat, without fat or tendons in a glass container fitted with a lid.
(4) Pour 1300.0 cc. of distilled water over the meat.
(5) Store in a cool place until the next day.
(6) Filter thru 4 thicknesses of cloth and press the remaining meat to obtain as much fluid as possible.
(7) Pour the filtrate into a kettle, add 10.0 g. peptone (siccum), 5.0 g. NaCl and the whites of two eggs, beaten up in 2 or 3 volumes of water.
(8) Boil under a gas flame for 15 minutes.
(9) Adjust to faint alkalinity, using phenolphthalein as an indicator, and the end point being a faint red color.
(10) Pour into an iron kettle, add 100.0 cc. distilled water, boil strongly for 5 minutes and filter.
(11) Add 1 liter of (10) to (2).
(12) Boil until there remains only 1 liter of medium.
(13) Filter thru a hot funnel.
(14) Distribute into test tubes and steam on 3 successive days for one hour.

(c) Frothingham (1895).
(1) Add one pound of finely chopped lean meat to 1000.0 cc. water, and let stand for about one hour.
(2) Heat in the water bath to about 60°C. for 3 hours.
(3) Boil 30 minutes.
(4) Filter and when cool make slightly alkaline (3.0 to 5.0 g. of Liebig's meat extract may be substituted for the meat infusion).
(5) Dissolve 5.0 g. NaCl and 10.0 g. dried peptone in (4).
(6) Boil 15 minutes, preferably over a free flame.
(7) Add 10.0 to 20.0 g. agar to (6) and boil until dissolved.
(8) Neutralize if necessary and add glycerol if desired.
(9) Filter.
(10) Sterilization not specified.

(d) Frothingham (1895).
(1) Add one pound of finely chopped lean meat to 1000.0 cc. of water and allow to stand from 12 to 24 hours in a cool place.
(2) Strain thru a cheese cloth or a coarse towel and squeeze in a meat press or by twisting the ends of the cloth until 1000.0 cc. of the meat juice is obtained. Make up to 1000.0 cc. by the addition of water if necessary. (Three to 5.0 g. of Liebig's meat extract may be substituted for the meat infusion.)
(3) Dissolve 5.0 g. NaCl and 10.0 g. dried peptone in (2).
(4) Boil 15 minutes either in steam or over a flame.
(5) Dissolve 10.0 to 20.0 g. of agar in (5) by boiling.
(6) Neutralize, if necessary and add glycerol if desired.
(7) Boil from 45 minutes to an hour.
(8) Filter. The filtrate should be clear. If not add the yolks of 2 eggs, mix rapidly and boil for a quarter to a half hour. Filter.
(9) Sterilization not specified.

(e) Ravenel (1898–1900).
(1) Chop 500.0 g. meat and mix with 500.0 cc. water.
(2) Place in cool place over night and then strain.
(3) Chop agar-agar and add to 500.0 cc. of water.
(4) Put (3) in autoclave and run up to 2 atmospheres of pressure.
(5) Cool below 100°C. before opening.
(6) Cool (5) to 75°C.
(7) Mix (2) and (6).
(8) Add 1.0% dried peptone and 0.5% salt.
(9) Boil 3 to 5 minutes.

(10) Neutralize (indicator not specified).
(11) Filter.
(12) Sterilization not specified.

(f) Jensen (1898) studied denitrification using the following medium:
(1) Prepare a meat infusion from 500.0 g. of meat and 1000.0 cc. of water.
(2) Dissolve 5.0 g. NaCl, 10.0 g. peptone and 15.0 g. agar in (1).
(3) Adjust to a slight alkalinity with soda.
(4) Method of sterilization not given.

(g) Committee A. P. H. A. (1899).
(1) Macerate one part finely chopped lean meat with 2 parts distilled water in the ice box for 18 to 24 hours, stirring occasionally.
(2) Strain cold thru a fine cloth.
(3) Add 1.0% peptone and 0.5% NaCl to the filtrate. Heat until solution is complete.
(4) Add NaOH until the reaction is slightly alkaline (practically neutral) to phenolphthalein.
(5) Heat on a water bath for 30 minutes and boil for 5 minutes over a free flame.
(6) Filter while hot thru paper or cotton and cloth, and add 1.0 to 2.0% agar to filtrate. Dissolve by boiling or autoclaving.
(7) Add N/1 HCl to the filtrate to obtain the desired reaction (+1.5).
(8) If the medium is clear distribute in tubes or flasks. If not clear, clarify by adding the white of one egg to the agar cooled to 50 to 60°C., and then boil vigorously. Filter.
(9) Sterilize either by the fractional or continuous method.

(h) Ravenel (1899).
(1) Mix 500.0 g. of freshly chopped meat with 500.0 cc. of water.
(2) Allow to stand in a cool place over night.
(3) Strain thru a towel.
(4) Chop agar into small pieces and add to 500.0 cc. water.
(5) Put in an autoclave and run the pressure up to two atmospheres (135.1°). Turn out the flame.
(6) Cool the autoclave at 100°C. before opening.
(7) Cool to 75°C.
(8) Mix (7) and (3).
(9) Add 10.0 g. peptone and 5.0 g. NaCl to (8).
(10) Boil for 3 to 5 minutes.
(11) Neutralize (indicator not specified).
(12) Filter.
(13) Sterilization not specified.

(i) Thalmann (1900) used the following medium for the isolation of gonococci:
(1) Cut lean beef into small pieces in a meat cutting machine.
(2) Add a double weight of distilled water to (1).
(3) Boil for 15 minutes stirring continually with a glass rod.
(4) Make up the loss of water and filter thru a filtering cloth.
(5) Add 1.0% peptone (siccum) and 0.5% NaCl.
(6) Boil.
(7) Make up the volume of water lost.
(8) Cool (in a closed container) and filter.
(9) Distribute in 300 to 500.0 cc. portions in clean flasks with patented sealers.
(10) Sterilize in streaming steam for one hour.
(11) Add 1.5% agar to (10).
(12) Bring to a boil on a concentrated salt solution bath and boil for 45 minutes shaking often.
(13) Take 30.0 cc. of (12) add a drop of alcoholic phenolphthalein solution and add N/1 sodium solution until a red coloration is formed.
(14) Estimate the amount (12) and calculating from (13) add $\frac{2}{3}$ the amount of sodium solution required to give neutralization.
(15) Keep in the autoclave or hot water for 15 minutes.
(16) Filter.
(17) Tube.
(18) Sterilize (exact method not specified).

(j) Walbaum (1901).
(1) Method of preparation of meat infusion not given.

(2) Add 10.0 g. of finely cut agar to 1000.0 cc. of (1).
(3) Allow to stand until the agar is completely soaked up. The time required depends on the type of agar used.
(4) Boil for about 45 minutes with constant stirring.
(5) Prepare a solution of 5.0 g. NaCl and 10.0 g. peptone by heating at about 70°C. (material in which the peptone and NaCl is to be dissolved not specified, whether it be water or of some of the infusion).
(6) Add distilled water to (4) to make up the loss due to evaporation.
(7) Add (5) to (6).
(8) Neutralize and boil again for a short time.
(9) Filter thru a single folded filter paper, placing the funnel and agar in an autoclave (requires 20–40 minutes depending on the kind of agar).
(10) Sterilization not specified.

(k) Migula (1901).
(1) Mix 500.0 g. of finely chopped lean beef with one liter of water, and allow to stand in the ice box for 12 to 24 hours.
(2) Press the liquid thru a towel and make up the volume to one liter.
(3) Boil in the steam cooker for 30 minutes.
(4) The infusion may be boiled for a hour before removing the meat and then filtered thru paper. If the liquid is still red, boil again for 15 minutes.
(5) Filter when cold to remove fat.
(6) Dissolve 15 0 g. agar, 0.5% NaCl and 1.0% Witte's peptone in (5) by boiling in a paraffin oven or an autoclave.
(7) Neutralize carefully by the addition of concentrated Na_2CO_3 solution until litmus paper is colored violet.
(8) Add soda solution as desired. Generally 10.0 cc. of a 15.0% soda solution is added per liter.
(9) Place the agar in a tall narrow flask and boil in the steamer for one hour.
(10) Cool to 90°C. and allow to remain at that temperature for several hours.
(11) Decant the clear agar and filter thru a folded filter paper. Filter at 90°C. The sediment may be filtered thru another filter paper.
(12) 1.0 to 2.0% glucose may be added.
(13) Distribute in tubes or flasks.
(14) Boil for one hour to sterilize.

(l) Thoinot and Masselin (1902).
(1) Macerate 500.0 g. of lean beef with 1 liter of water for several hours.
(2) Pass thru a linen cloth and express the juice from the meat.
(3) Add 10.0 g. peptone and 5.0 g. NaCl.
(4) Boil and filter.
(5) Make slightly alkaline by the addition of soda.
(6) Add 15.0 g. of finely chopped agar and heat until dissolved. Stir constantly.
(7) Pass thru a sieve.
(8) Cool to 55°C. and add the white of an egg beaten up in 500.0 cc. of water. Mix well.
(9) Heat to 120°C. for 45 minutes.
(10) Filter thru paper (hot filtration).
(11) Distribute in tubes.
(12) Heat at 115°C. for 20 minutes.

(m) Frost (1903).
(1) Add 15.0 g. of agar to 500.0 cc. of water and dissolve by heating to 120°C. in the autoclave, closing off the gas and allowing to cool or boil until the agar is dissolved (about 30 minutes) and make up the loss of water due to evaporation.
(2) Free 500.0 g. of lean beef from fat and connective tissue and mince.
(3) Add 500.0 g. of water to (2) and set in the ice chest for 12 to 24 hours.
(4) Squeeze thru a cloth and add distilled water to the filtrate to make 500.0 cc.
(5) Add 10.0 g. of peptone and 5.0 g. NaCl.
(6) Heat until solution is complete.
(7) Neutralize to phenolphthalein.
(8) Cool to 60°C.

(9) Add (1) to (8).
(10) Boil until albumin is coagulated and floats in the clear liquid. Restore to weight.
(11) Readjust the reaction if necessary.
(12) Add 0.5% of normal HCl.
(13) Filter thru cotton supported on a coil of wire using a suction pump to hasten filtration.
(14) Tube.
(15) Sterilize in steam for 15 minutes on 3 successive days or in the autoclave for 20 minutes at 120°C. Slant some of the tubes after final sterilization.

(n) Frost (1923).
(1) Add 15.0 g. agar to 500.0 cc. of water and dissolve by heating to 120°C. in the autoclave, closing off the gas, and allowing to cool or boil until the agar is dissolved (about 30 minutes) and make up the loss of water due to evaporation.
(2) Free 500.0 g. of lean beef from fat and connective tissue and mince.
(3) Add 500.0 cc. of distilled water.
(4) Place in a vessel for cooking and then cook for 30 minutes at 70°C.
(5) Filter thru paper and make up the volume to 500.0 cc.
(6) Add 10.0 g. of peptone and 5.0 g. of NaCl.
(7) Heat until solution is complete.
(8) Neutralize to phenolphthalein.
(9) Cool to 60°C.
(10) Add (1) to (9).
(11) Boil until albumin is coagulated and floats in the clear fluid. Restore the weight.
(12) Readjust the reaction if necessary.
(13) Add 0.5% of normal HCl.
(14) Filter thru cotton supported on a coil of wire using a suction pump to hasten filtration.
(15) Tube.
(16) Sterilize in steam for 15 minutes on 3 successive days or in the autoclave for 20 minutes at 120°C. Slant some of the tubes after sterilization.

(o) Smith (1905).
(1) Add 1000.0 cc. distilled water to 500.0 g. of finely minced lean beef.
(2) Infuse for 24 hours in the ice chest or heat for one hour in the water bath at 55°C.
(3) Boil for 60 minutes in the steamer or in a covered dish.
(4) Filter thru a clean cloth in a meat press.
(5) Cool and remove the fat by filtering thru S and S filter paper.
(6) Make up the volume to 1000.0 cc. by the addition of water.
(7) Add 1.0% Witte's peptone and 0.5% NaCl.
(8) Steam for one-half hour.
(9) Filter.
(10) Cool.
(11) Adjust the reaction.
(12) Steam again for 30 minutes.
(13) Filter.
(14) Add 10.0 g. powdered agar to the filtrate.
(15) Steam for 30 minutes and cool to 58°C.
(16) Add the whites of 2 eggs (beaten thoroly and neutralized to litmus by dilute HCl).
(17) Steam one hour and filter while hot thru S & S filter paper which has been thoroly warmed with boiling distilled water. The agar may be filtered thru absorbent cotton.
(18) Tube.
(19) Sterilize by heating in the autoclave at 110°C. for 10 minutes or by short steamings in the steamer on each of 3 successive days.

(p) Guillemard (1906) cultivated anaerobes on the following medium:
(1) Mix 500.0 g. of chopped meat, 10.0 g. peptone and 5.0 g. NaCl in 1000.0 cc. water.
(2) Bring to a boil and boil 10 minutes.
(3) Filter while hot.
(4) Allow to cool for 12 hours.
(5) Filter while cold.
(6) The solution is clear and of an acid reaction.
(7) Make up to a liter if necessary.
(8) Add a piece of red litmus paper.

(9) Then add a 36°B. soda solution drop by drop until the red litmus is turned blue.
(10) Add 10.0 g. agar and dissolve by heating.
(11) Heat at 115 to 120° in the autoclave.
(12) A precipitate forms which completely clears the medium.
(13) Filter and distribute in tubes.
(14) Sterilize at a temperature lower than in (11).

(q) Walbaum (1910).
(1) Method of preparation of meat infusion not given.
(2) Add 100.0 g. of finely cut agar to 1000.0 cc. of (1).
(3) Place in the autoclave for 30 minutes at 0.5 atmosphere extra pressure.
(4) Prepare a solution of 10.0 g. peptone and 5.0 g. NaCl by heating at about 70°C. (Material in which the peptone and NaCl is to be dissolved not specified, whether it be some of the infusion or water).
(5) Place the agar, after autoclaving is completed, on a free flame, and add the distilled water to make up the loss in weight due to evaporation.
(6) Add (4) to (5).
(7) Neutralize and boil again for a short time.
(8) Filter thru a single or double thickness of filter paper. (Requires about 10 minutes.)
(9) Sterilization not specified.

(r) Abel (1912).
(1) Chop 500.0 g. of fat free meat and add to a liter of water at 50°C.
(2) Keep at 50°C. for 30 minutes and then boil for 30 to 45 minutes.
(3) Filter or strain the fluid from the heat.
(4) Make up the fluid to 1 liter.
(5) Place 20 g. agar in a 3 liter flask and add 500.0 cc. of tap water and 2.5 cc. of glacial acetic acid.
(6) Allow (5) to soak 15 minutes and drain carefully.
(7) Wash the agar thoroly with 4 lots of water.
(8) After the last washing allow the agar to drain for 10 minutes.
(9) Add 500.0 cc. of (4), 10.0 g. peptone and 5.0 g. NaCl to the washed agar.
(10) Autoclave at 115°C. for 30 minutes or in the steamer for 90 minutes.
(11) Make (10) up to a liter by the addition of (4).
(12) Make slightly alkaline to litmus by the addition of KOH.
(13) Cool to 60°C. and add the beaten white of an egg.
(14) Heat in the autoclave for 45 minutes or in the steamer, for 90 minutes.
(15) Filter thru moistened filter paper, using a hot water funnel.
(16) Tube.
(17) Steam for 30 minutes on each of 2 successive days.

(s) Abel (1912).
(1) Chop 500.0 g. of fat free meat and add to a liter of water at 50°C.
(2) Keep at 50°C. for 30 minutes and then boil for 30 to 45 minutes.
(3) Filter or strain the fluid from the meat.
(4) Make up the fluid to 1 liter.
(5) Soak 1.5 or 2.0% agar in (4) for several hours.
(6) Add 1.0% Witte or Chapoteaut's peptone and 0.5% NaCl.
(7) Heat in the steamer until solution is complete.
(8) Neutralize to litmus if necessary.
(9) Heat in the steamer for 15 to 30 minutes.
(10) Filter thru cotton-wool to clarify. The agar may be allowed to solidify in the steamer in straight walled vessels and cut away the bottom opaque layer.
(11) Sterilize on each of 3 successive days in the steamer or autoclave at 120°C. for 15 minutes.

(t) Wilcox (1916) produced toxin by growing *B. tetani* on the following medium:
(1) Dissolve 5.0 g. agar, 10.0 g. Witte's peptone and 5.0 g. NaCl in 1000.0 cc. veal infusion.

(2) Adjust reaction so that it is neutral to phenolphthalein.
(3) Tube in 8 to 10.0 cc. quantities.
(4) Autoclave at 15 pounds for ½ hour.
(5) To transfer cultures, a semi-solid agar culture is melted and 1.0 cc. portions transferred to fresh semi-solid melted agar tube.

(u) Meier (1918).
(1) Boil 500.0 g. of fat and tendon free beef in 1 liter of water.
(2) Filter.
(3) Dissolve 15.0 g. agar, 10.0 g. Witte's peptone and 5.0 g. NaCl in the filtrate.
(4) Neutralize by the addition of KOH. Add KOH until turmeric paper is turned quite weakly brownish red.
(5) Sterilization not specified.

(v) Harvey (1921–22).
(1) Mince finely fat-free beef.
(2) Add 500.0 to 1000.0 cc. distilled water or clear tap water.
(3) Heat the mixture 20 minutes over a free flame at a temperature not exceeding 50°C.
(4) Skim off fat floating on the surface.
(5) Raise the temperature to boiling point.
(6) Boil 10 minutes.
(7) Pour the mixture onto a wet, thick, clean cloth.
(8) Collect the fluid which drains thru the cloth together with that obtained by squeezing the meat in the cloth.
(9) Add 10 g. peptone 5.0 g NaCl.
(10) Mix fiber agar 1 part, glacial acetic acid 0.05 parts, and water 2 parts.
(11) Allow the agar to soak in the acidified water 15 minutes.
(12) Remove the fiber agar and wash thoroly with water until quite free from any trace of acid reaction to litmus paper.
(13) Squeeze in a cloth to get rid of excess of water.
(14) Add (13) to (9).
(15) Steam gently 2½ hours to bring the agar thoroly into solution.

Note: Or heat 45 minutes at 118°C.

(16) Bring the volume up to 1000.0 cc. by the addition of hot water.
(17) Estimate and adjust the reaction to a definite pH value, or to faintly alkaline to litmus or 1.0% acid to phenolphthalein.
(18) Steam 30 minutes.
(19) Filter, while hot, thru well-wetted, thick filter paper or two paper or two layers of absorbent cotton wool, by placing filter funnel, stand, and receptacle for filtrate in the steam sterilizer and steaming till filtration is complete.

Note: If filtration thru paper is not sufficient to give a clear medium, clarify with white of egg or other clearing agent.

(a) Beat up the white of one or two eggs along with the crushed shells in about 20.0 cc. water.

Note: Raw meat juice, 15.0 cc. per liter of medium may be substituted for white of egg.

(b) Add to the medium little by little before filtration and at a temperature not exceeding 60°C.
(c) Stir to mix.
(d) Steam 30 minutes.
(e) Remove from steamer and shake up well to mix.
(f) Steam again 15 minutes.
(g) Filter in the steamer thru thick filter paper, or thru two layers of absorbent cotton.
(h) Refilter, if necessary, the first portion of the filtrate.

(20) Distribute into flasks on test tubes.

(w) Harvey (1921–22).
(1) Mince finely fat-free beef.
(2) Add 500.0 g. to 1000.0 cc. distilled water or clear tap water.
(3) Heat the mixture 20 minutes over a free flame at a temperature not exceeding 50°C.
(4) Skim off fat floating on the surface.
(5) Raise the temperature to boiling point.
(6) Boil 10 minutes.
(7) Pour the mixture on to a wet, thick, clean cloth.

(8) Collect the fluid which drains thru the cloth together with that obtained by squeezing the meat in the cloth.
(9) Filter the fluid collected thru well-wetted, thick filter paper.
(10) Add to the filtrate—10.0 g. peptone, 5.0 g. sodium chloride.
(11) Steam or boil 45 minutes.
(12) Bring the volume up to 1000.0 cc. by the addition of water.
(13) Cut up 20.0 g. fiber agar into small pieces.
(14) Add to 1000.0 cc. hot bouillon (12).
(15) Bring the agar into solution with heat after having been allowed to soak 2 hours in the hot bouillon.
(16) Estimate and adjust the reaction to a definite pH value or faintly alkaline to litmus or 1.0% acid to phenolphthalein.
(17) Steam 30 minutes.
(18) Clarify and filter.
  (a) Beat up the white of one or two eggs along with the crushed shells in about 20.0 cc. water.
    NOTE: Raw meat juice, 15.0 cc. per liter of medium, may be substituted for white of egg.
  (b) Add to the medium little by little before filtration and at a temperature not exceeding 60°C.
  (c) Stir to mix.
  (d) Steam 30 minutes.
  (e) Remove from steamer and shake well to mix.
  (f) Steam again 15 minutes.
  (g) Filter in the steamer thru thick filter paper, or thru two layers of absorbent cotton wool.
  (h) Refilter, if necessary, the first portion of the filtrate.
(19) Distribute into test tubes.
(20) Sterilize in the steamer or autoclave.

(x) Harvey (1921–22).
(1) Mince 250.0 g. fat-free beef with 500.0 cc. of water.
(2) Heat the mixture 20 minutes over a free flame, at a temperature not exceeding 50°C.
(3) Skim off fat floating on the surface.
(4) Raise the temperature to boiling point.
(5) Boil 10 minutes.
(6) Pour the mixture on to a wet, thick, clean cloth.
(7) Add 2.5 g. NaCl to the filtrate.
(8) Bring the volume up to 1000.0 cc. by the addition of water.
(9) Estimate and adjust the reaction.
(10) Steam 30 minutes.
(11) Filter, while hot, thru well-wetted, thick filter paper.
(12) Place 15.0 g. fiber agar in 500.0 cc. tap water.
(13) Wash the agar well by squeezing it thru the hands.
(14) Decant and reject the dirty water.
(15) Replace with the same amount of clean tap water.
(16) Heat over a free flame with constant stirring to dissolve the agar.
(17) Add peptone 5.0 g., sodium chloride 2.5 g.
(18) Add to 500.0 cc. of meat extract (11), 10.0 g. egg albumin which has been made into a paste or suspension with a little of the meat infusion.
(19) Add the dissolved agar slowly to the meat extract thus prepared, with constant stirring.
(20) Steam 60 minutes or heat 45 minutes at 120°C.
(21) Bring the volume to 1000.0 cc. by the addition of water.
(22) Estimate and adjust the reaction.
(23) Boil 15 minutes over a free flame.
(24) Make up for any loss of volume by addition of water.
(25) Filter, while hot, thru well-wetted, thick filter paper by placing filter funnel, stand, and receptacle for filtrate in the steamer and steaming till filtration is completed.
(26) Distribute filtrate into flasks or test tubes.
(27) Sterilize in the steamer or autoclave.

(y) Harvey (1921–22).
(1) Soak 10.0 g. purified agar (see medium 1401 for preparation) in 500.0 cc. of Harvey's Infusion

Broth (see variant (bb) medium 779) for 2 hours.
(2) Bring the agar into solution by heating.
(3) Sterilization not specified.

(z) Harvey (1921–22).
(1) Add 500.0 cc. of water to 500.0 cc. of the variant given by Harvey above.

(aa) Abbott (1921).
(1) Add 500.0 g. chopped lean beef to 1 liter of water and soak for 24 hours, kept at ice box temperature.
(2) Strain thru a coarse towel and press until a liter of fluid is obtained.
(3) Dissolve 1.0% peptone, and 0.5% NaCl in (2).
(4) Dissolve 1.0 to 1.5% agar in (3) by boiling in a porcelain lined iron vessel. Add 250.0 to 300.0 cc. of water and boil until this volume of water has evaporated, leaving one liter volume.
(5) Place the vessel in a large dish of cold water.
(6) Stir the agar constantly until cooled to 68 to 70°C.
(7) Add the white of one egg which has been beaten up in about 50.0 cc. of water (a 10.0% dry albumin solution may be used).
(8) Mix (7) thoroly with (6).
(9) Allow to boil slowly for 30 minutes. Do not allow the volume of the liquid to fall below the liter mark.
(10) Filter thru a heavy folded filter paper.
(11) Sterilize by steam (method not given).

(bb) Dopter and Sacquépée (1921).
(1) Add 1000.0 cc. of water to 500.0 g. of finely chopped fat and tendon free beef.
(2) Allow to stand in ice box for 12 hours, or heat at 50 to 55° for 30 minutes.
(3) Heat slowly to boiling.
(4) Boil slowly for 10 minutes, stirring constantly.
(5) Press the liquid thru a clean cloth.
(6) Add 20.0 g. peptone and 5.0 g. NaCl to 1000.0 cc. of the filtrate and dissolve by shaking.
(7) Filter thru a wetted filter paper.
(8) Make slightly alkaline to litmus by the addition of NaOH or Na_2CO_3.
(9) Soak 20.0 g. of agar in (8) for several hours.
(10) Boil until solution is complete, stirring constantly.
(11) Test, and readjust the reaction if necessary.
(12) Cool to 60°C.
(13) Add the white of one egg beaten up in 50 to 100.0 cc. of water.
(14) Add (13) to (12).
(15) Heat in the autoclave at 118° for 15 minutes.
(16) Filter while hot thru paper.
(17) Tube.
(18) Sterilize at 115° for 20 minutes.

(cc) Pitfield (1922).
(1) Cover 500.0 g. of finely cut fat free beef with 1000.0 cc. of water.
(2) Shake well and place on ice over night.
(3) Squeeze out the fluid by means of a cloth and make up the volume to 1 liter.
(4) Inoculate with a culture of the colon bacillus.
(5) Allow to stand at room temperature over night.
(6) Boil and add 10.0 g. Witte's peptone and 5.0 g. NaCl.
(7) Weigh the saucepan and contents and heat to 60°C.
(8) Make up the loss in weight by the addition of water.
(9) Neutralize to litmus.

(dd) Klimmer (1923).
(1) Preparation of meat water not given.
(2) Soak the agar in water from 1 to 12 hours.
(3) Pour off the water and press the agar free from liquid.
(4) Add (3) to (1) and boil until solution is complete (steamer or free flame).
(5) Make up the loss in weight by the addition of water.

(6) Add 10.0 g. peptone and 5.0 g. NaCl to (5).
(7) Neutralize.
(8) Boil.
(9) Readjust the reaction if necessary. Alkaline agar may be prepared by adding 7.0 cc. of normal soda solution to a liter of agar neutral to litmus.
(10) Filter.
(12) Distribute as desired.
(13) Sterilize by steaming for 40 minutes, on one day, or for 15 minutes on each of 3 successive days.

(ee) Park, Williams and Krumwiede (1924).
(1) Dissolve 10.0 g. peptone and 5.0 g. NaCl in 1000.0 cc. infusion broth (see variant (ll) medium 779) by heating to 50°C.
(2) Add 15.0 g. agar to (1) and dissolve by heating in the autoclave at 10 to 15 pounds pressure for 30 minutes, or by boiling over the free flame.
(3) If boiled over a free flame, make up the loss in weight by the addition of water.
(4) Adjust the reaction.
(5) Cool to 50°C. and add one egg.
(6) Heat in the autoclave at 10 to 15 pounds for 30 minutes or Arnold sterilizer for 1 hour. Filter.
(7) Test the reaction and adjust if necessary.
(8) If more than 0.2% normal soda is required per liter, heat again for 10 minutes.
(9) Filter thru cotton.
(10) Distribute in tubes or flasks.
(11) Sterilize at 15 pounds pressure for 30 minutes.

(ff) Park, Williams and Krumwiede (1924).
(1) Dissolve 10.0 g. peptone, 5.0 g. NaCl and 15.0 g. agar in infusion broth. (See variant (ll) medium 779) by heating in the autoclave at 10 to 15 pounds pressure for 30 minutes or by boiling over the free flame.

Remainder of preparation the same as variant (ee) above.

(gg) Park, Williams and Krumwiede (1924).
(1) Prepare double strength infusion broth, using double the amount of beef or veal. (See infusion broth medium 779, variant (kk) or (ll) for the method.)
(2) Add double the amount of peptone, (20.0 g. per liter) and NaCl (10.0 g. per liter) to (1).
(3) Adjust the reaction.
(4) To an equal quantity of water (1000.0 cc.) add a double quantity (30.0 g.) of agar and dissolve.
(5) Cool the agar below 50°C.
(6) Mix equal parts of (3) (double strength infusion broth) and (5) (3.0% agar).
(7) Test the reaction and adjust if necessary.

Remainder of preparation as from step (5) variant (ee) above.

References: Jacobi (1888 p. 536), Schultz (1891 pp. 57, 60), Frothingham (1895 p. 55), Ravenel (1898–1900 p. 89), Jensen (1898 p. 406), Committee A. P. H. A. (1899 p. 77), Ravenel (1899 p. 606), Thalmann (1900 p. 829), Walbaum (1901 p. 796), Migula (1901 p. 17), Thoinot and Masselin (1902 p. 33), Frost (1903 p. 16), Smith (1905 p. 195), Guillemard (1906 p. 157), Walbaum (1910 p. 796), Abel (1912 pp. 17, 18), Wilcox (1916 p. 333), Meier (1918 p. 435), Harvey (1921–22 pp. 66, 70, 71), Abbott (1921 p. 129), Dopter and Sacquépée (1921 p. 125), Pitfield (1922 p. 117), Klimmer (1923 p. 193), Park, Williams and Krumwiede (1924 p. 117).

1662. Zettnow's Meat Infusion Agar

Constituents:

1. Water	5000.0 cc.
2. Meat (horse)	3000.0 g.
3. Agar	11.5 g.
4. $(NH_4)_2SO_4$	1.0 g.
5. Peptone	1.0 g.
6. KNO_3	1.0 g.

Preparation:

(1) Heat 3000.0 g. of horse (or other cheap fat-free meat) meat with 5000.0 cc. of tap water, or distilled water, to 60–65°C. for 1 to 1½ hours, stirring occasionally.

(2) Heat then to a boil stirring constantly.
(3) Filter thru a filtering cloth, washing the residue with one liter of water and squeeze out the residue.
(4) Distribute into glass flasks and sterilize twice before use. (This constitutes a stock meat infusion.)
(5) For the preparation of one liter of agar medium add 11.5 g. fibre agar to 500.0 g. water. Allow the agar to grow and swell in the water.
(6) Add 8.0 g. ovi albumin (siccum) to about 40.0 cc. of water. It dissolves slowly.
(7) Place 1000.0 g. of (4) (meat infusion) in a weighed kettle.
(8) Add 250–300.0 cc. water to (7) and 1.0 g. peptone.
(9) Heat nearly to boiling and neutralize with NaOH until litmus paper becomes strongly blue.
(10) Add (5) (the soaked agar) to (9) and boil over a free flame to dissolve the agar. Do not over heat.
(11) Add 1.0 g. $(NH_4)_2SO_4$ and 1.0 g. KNO_3 to (10).
(12) Weigh and add distilled water until the contents of the kettle weigh 1000.0 g.
(13) Cool to 50–55°C.
(14) Add (6) (albumin solution) or the whites of two eggs and place in a steamer for 50–60 minutes.
(15) Filter thru a hot water funnel. (May use a folded felty, so-called coffee filter paper.)

Sterilization: Method not given.

Use: Cultivation of *Spirillum undula majus* and other spirilla.

Reference: Zettnow (1895 p. 394).

1663. Besson's Phosphate Infusion Agar

Constituents:

1. Water	1000.0	cc.
2. Beef	500.0	g.
3. Peptone	10.0	g.
4. Agar	20.0	g.
5. Sodium phosphate	1.0	g.
6. NaCl	5.0	g.

Preparation:

(1) Remove all fat and tendons from beef and chop into small pieces.
(2) Allow 500.0 g. of (1) to macerate with 1000.0 cc. cold water for 6 hours, or if one wishes to remove the sugar, 12 hours at 37°C.
(3) Place in an enamelled pot and bring slowly to a boil.
(4) Boil for 10 minutes.
(5) Throw on a thick cloth and press the meat free from juice.
(6) Filter the juice thru moistened paper.
(7) Add 10.0 g. of Chapoteaut's or Defresne peptone, 5.0 g. NaCl and about 1.0 g. of sodium phosphate.
(8) Boil stirring constantly until solution is complete.
(9) Neutralize or make slightly alkaline to litmus by the addition of soda solution.
(10) Soak 20.0 g. of chopped thread agar in cold water for several hours.
(11) Heat (10) and (9) at 100°C. until the agar is dissolved.
(12) Readjust the reaction if necessary.
(13) Allow to cool to 55 or 60°C.
(14) Beat the white of an egg in 100.0 cc. of water and add to (13).
(15) Mix well.
(16) Autoclave at 120°C. for one hour.
(17) Filter thru a moistened Chardin filter using a hot water funnel.
(18) Tube.

Sterilization: Sterilize at 115° for 20 minutes.

Use: General culture medium.

Variants:

(a) The agar may be treated with a 6 to 100 solution of HCl (500.0 cc. water to 30.0 cc. HCl for 24 hours and then with a 5.0% ammonia solution. Then thoroly wash the agar with water.
(b) Besson also used 20.0 g. peptone instead of 10.0 g.

Reference: Besson (1920 p. 41).

1664. Smith's Infusion Agar

Constituents:

1. Distilled water	2000.0	cc.
2. Beef	1000.0	g.
3. Peptone (Merck, brown)	20.0	g.
4. Agar	20.0	g.

Preparation:

(1) Soak finely chopped fat free beef with 2000.0 cc. distilled water for some hours and then slowly raise to 65°C. on the water bath.
(2) Finally steam and filter.

(3) Add 20.0 g. Merck's brown peptone and 20.0 g. agar.
(4) Steam (time not specified).
(5) Cool and add the whites of 10 eggs to clarify.
(6) Resteam and filter.
(7) Neutralize the egg albumin with HCl and then render the medium alkaline with NaOH.

Sterilization: Not specified.

Use: General culture medium. Smith cultivated *Pseudomonas campestris* (Pammel) on the medium and reported that the colonies developed moderately fast. They were circular or nearly so, thin and well defined margin pale yellow wet and shining. Streaked cultures tended to spread. In plates or streaks crystals of ammonium magnesium phosphate formed after several days.

(a) Committee A. P. H. A. (1901 recommended the following medium to be used for bacterial counts in water analysis.
  (1) Boil 15.0 g. thread agar in 500.0 cc. water for half an hour and make up weight to 500.0 g. or digest for 10 minutes in the autoclave at 110°C. Let this cool to about 60°C.
  (2) Infuse 500.0 g. lean meat 24 hours with 500.0 cc. of distilled water in refrigerator.
  (3) Make up any loss by evaporation.
  (4) Strain infusion thru cotton flannel.
  (5) Weigh filtered infusion.
  (6) Add 2.0% Witte's peptone.
  (7) Warm on water bath, stirring till peptone is dissolved and not allowing the temperature to rise above 60°C.
  (8) Neutralize.
  (9) To 500.0 g. of the meat infusion add 500.0 cc. of the 3.0% agar, keeping the temperature below 60°C.
  (10) Heat over boiling water, (or steam) bath for 30 minutes.
  (11) Restore loss by evaporation.
  (12) Titrate, after boiling one minute to expel carbonic acid.
  (13) Adjust reaction to +1.0% by adding normal hydrochloric acid or sodium hydrate as required.
  (14) Boil 2 to 5 minutes over a free flame, stirring constantly.
  (15) Make up loss due to evaporation.
  (16) Filter thru absorbent cotton and cotton flannel, passing the filtrate thru the filter until clear.
  (17) Titrate and record the final reaction.
  (18) Tube in 7.0 cc. quantities.
  (19) Sterilize 15 minutes in the autoclave at 110° or for 30 minutes in streaming steam on 3 successive days.
  (20) Store in the ice chest in a moist atmosphere, to prevent evaporation.

(b) Committee A. P. H. A. (1905) recommended the same method of preparation as in 1901, but sterilized the medium by heating for 5 minutes at 120°C. or for 30 minutes in streaming steam on 3 successive days.

(c) Committee A. P. H. A. (1905) prepared a basal medium as given under variant (b) above and added 10.0 g. of glucose or lactose just before sterilization. The reaction is then adjusted to neutral to phenolphthalein. If medium is to be used in tubes add sterilized azolitmin solution (amount not specified) just before final sterilization. If medium is to be used in Petri dishes, add sterilized azolitmin solution (amount not specified) just before the medium is to be poured into the plate.

(d) Committee A. P. H. A. (1909) recommended that the medium be adjusted to +1 5 instead of +1.0 as in variant (a). Sterilize in the autoclave for one hour at 15 pounds pressure or in streaming steam for 20 minutes on 3 successive days. To prepare lactose or glucose litmus agar, add 1.0% lactose or 1.0% glucose to the medium just before sterilization. The reaction is adjusted to neutral to phenolphthalein. Add the sterilized azolitmin to the medium just before sterilization if the agar is to be used in tubes. If to be used in plates, add the sterilized azolitmin solution to the Petri dishes when pouring plates.

- (e) Meier prepared a similar medium as follows:
  - (1) Boil 500.0 g. of fat and tendon free beef in 1 liter of water.
  - (2) Filter.
  - (3) Dissolve 15.0 g. agar, 10.0 g. peptone and 5.0, 20.0 or 40.0 g. of lactose or glucose in the filtrate.
  - (4) Neutralize by the addition of KOH. Add KOH until turmeric paper is turned quite weakly brownish red.
  - (5) Sterilization not specified.

References: Smith (1897 p. 480), Committee A. P. H. A. (1901 p. 384), (1905 p. 108), (1905 Sup. #1), (1909 p. 285), Meier (1918 p. 436).

1665. Francis' Basal Infusion Agar

Constituents:

1. Infusion agar (1.5%)	1000.0 cc.
2. Peptone (1.0%)	10.0 g.

Preparation:

- (1) Prepare a stock beef infusion agar using fresh beef, 1.0% agar and 1.0% peptone.
- (2) Adjust (1) to pH-7.6.
- (3) Add one of the added nutrients in the manner indicated below.
- (4) Incubate 24 to 48 hours to test sterility.

Sterilization: Method not given.

Use: Cultivation of *Bacterium tularense*.

Variants:

- (a) The author used 1.0% agar in preparation of media as indicated under added nutrients.
- (b) Francis adjusted the reaction of the stock agar to pH-7.3 when preparing serum glucose cystine agar, serum glucose cysteine hydrochloride agar, and cysteine hydrochloride agar.

Added nutrients: Francis prepared the media listed below as indicated.

- (a) Serum glucose agar. Add 1.0% glucose from a sterile 50.0% glucose solution and 5.0% serum to the melted agar cooled to 45°C.
- (b) Glucose blood agar. Substitute 5.0% defibrinated blood for serum in (a).
- (c) Blood agar. Same as (b) but add no glucose.
- (d) Mediums (a), (b) and (c) plus spleen tissue.
  - (1) Remove spleen from a healthy rabbit and cut into pieces 3 mm. in diameter under aseptic conditions.
  - (2) Rub one piece of (1) on each agar slant of (a), (b) or (c) and allow the spleen to remain on the surface of each slant just above the water of condensation.
- (e) Cystine agar. Add 0.02% cystine to solid sterile 1.0% stock agar and steam to melt agar and sterilize the cystine.
- (f) Spleen agar. Rub pieces of spleen over 1.0% stock agar slants in the manner as indicated under (d) (1) and (2) above.
- (g) Serum glucose cystine agar.
  - (1) Add 0.1% cystine and 1.0% glucose to solid sterile 1.0% stock agar.
  - (2) Steam to melt the agar and to sterilize the cystine and glucose.
  - (3) Cool to 50°C.
  - (4) Add 5.0% horse serum.
- (h) Serum glucose cysteine hydrochloride agar. Substitute cysteine hydrochloride for cystine in medium (g) above.
- (i) Cystine hydrochloride agar. Add 0.1% cystine hydrochloride and 1.0% glucose to sterile solid 1.0% stock agar and heat to melt the agar and sterilize the cysteine hydrochloride and glucose.

Reference: Francis (1922 p. 102, 987, 988) (1923 p. 1398).

1666. Deycke's Alkali Albuminate Agar

Constituents:

1. Water	1200.0 cc.
2. KOH (3.0%)	36.0 g.
3. Veal	1000.0 g.
4. NaCl	
5. Peptone	
6. Agar	

Preparation:

- (1) Digest 1000.0 g. of finely ground fat free veal in 1200.0 cc. of 3.0% KOH solution for 2 days at 37°C.
- (2) Heat (1) for one hour on a water bath at 60 to 70°C. until all the protein has dissolved.
- (3) Add HCl, carefully to precipitate the albuminates.
- (4) Collect on a filter.

(5) Suspend the precipitate in distilled water and add concentrated soda solution until part of the precipitate is brought into solution. Dissolve the remainder of the precipitate by prolonged heating in steam.
(6) Correct the reaction to a weak alkalinity.
(7) Dry 100.0 cc. of the solution and determine the per cent of dry material.
(8) Dilute (6) so that there is 2 to 3.0% solid materials present.
(9) Dissolve 1.0% peptone, 1.0% NaCl and agar (amount not given) to (8), method not given.
(10) Neutralize by Dahmen's method with 0.33% soda.

Sterilization: Not specified.

Use: Isolation of cholera bacilli from stools. Author reported that this medium eliminated practically all other organisms from stools.

Reference: Deycke (1893 p. 888).

1667. Harvey's Basal Infusion Agar

Constituents:

1. Infusion agar	1000.0 cc.

Preparation:

(1) Prepare infusion agar according to variant (v) medium 1661.
(2) Add one of the added nutrients to (1).

Sterilization: Not specified.

Use: Cultivation of *B. acnes* and anaerobes. Also used as an enrichment medium for the colon typhoid group.

Added nutrients: The author added one of the following:

(a)	Oleic acid	10.0 cc.
	glycerol	20.0 cc.
(b)	Glycerol	20.0 cc.
	oleic acid	1.0 cc.
(c)	Glucose	20.0 g.
	sulphin digotate	1.0 g.
(d)	Glucose	20.0 g.
(e)	Sodium formate	4.0 g.

Equal parts agar and 1.0% caffeine solution.

The combinations (a) and (b) were used to cultivate *B. acnes*, (c) and (d) used for the cultivation of anaerobes. Agar containing caffeine (e) was used as enrichment medium for members of the colon typhoid group.

Reference: Harvey (1921–22 pp. 87, 92, 111).

1668. Krasnow's et al. Meat Infusion Agar

Constituents:

1. Tap water	1000.0 cc.
2. Veal, Bacto	75.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Agar	20.0 g.

Preparation:

(1) Infuse 75.0 g. of Bacto Veal in 500.0 cc. of tap water in the Arnold sterilizer at 100°C. for two hours.
(2) Allow the coagulum thus formed to settle to the bottom of the container. Allow to cool very slowly.
(3) Strain thru a wire sieve.
(4) Dissolve 3 and 4 in (3).
(5) Adjust to pH 7.9.
(6) Steam in the Arnold for 15 minutes.
(7) Filter.
(8) Add an equal quantity of a 4.0% agar solution.

Sterilization: Sterilize in the autoclave for 45 minutes at 15 pounds pressure.

Use: Cultivation of streptococci.

Reference: Krasnow, Rivkin and Rosenberg (1926 p. 391).

1669. Dopter's Liver Infusion Agar (White)

Constituents:

1. Water	1000.0 cc.
2. Liver	500.0 g.
3. Agar (1.5%)	15.0 g.
4. Peptone (1.0%) (Witte, Chapoteau or Fairchild)	10.0 g.
5. NaCl (0.5%)	5.0 g.

Preparation:

(1) Mix 500.0 g. of well minced calf or beef liver with 1000.0 cc. of distilled water.
(2) Stir well and infuse over night in the ice box.
(3) Boil 5 minutes.
(4) Filter thru paper.
(5) Make up to original volume.
(6) Add 1.0% Witte, Chapoteau or Fairchild's peptone, 0.5% NaCl and 1.5% agar.
(7) Boil 20 minutes and adjust to +0.2 to phenolphthalein.
(8) Boil 5 minutes and filter.

Sterilization: Sterilize in the Arnold on three successive days.

Use: General culture medium. Also used to cultivate and carry stock meningococci cultures. Bronfenbrenner and Schlesinger reported that the medium gave a large amount of water of condensation. To keep a pure culture on this medium for several weeks, inoculate the water of condensation and tilt the tubes every day or so. Other investigators cultivated a variety of organisms as *B. welchii*, *B. chauvoei*, the gonococcus, etc., on similar media.

Variants:

(a) Bronfenbrenner and Schlesinger prepared the medium as follows:
  (1) Prepare a liver infusion using 1000.0 g. liver to 1000.0 cc. water. The extract is collected thru a single layer of cheese cloth. Boil and stir continually (Time not given).
  (2) Strain thru a single layer of cheese cloth and a thin layer of cotton.
  (3) Do not adjust the reaction, and sterilize at 10 pounds pressure for 10 minutes.
  (4) Prepare a 3.0% agar solution in water (30.0 g. agar to 1000.0 cc. water.
  (5) Filter thru cotton.
  (6) Add 2.0% (20.0 g.) peptone and 1.0% (10.0 g.) NaCl.
  (7) Do not adjust the reaction.
  (8) Mix equal parts of sterile (3) and sterile (7) while hot.
  (9) Tube in sterile tubes.

(b) Heller isolated *B. Welchii*, *B. oedematiens* and other soil anaerobes on the following medium:
  (1) Grind 250.0 g. beef liver and infuse with 4 parts water over night. Boil and strain.
  (2) Add 15.0 g. peptone, 5.0 g. NaCl and 2.0 g. Agar.
  (3) pH 7.2 (faintly alkaline to litmus).
  (4) Tube in deep tubes.
  (5) Method of sterilization not given. He reported that if large amount of H_2O be produced, add KNO_3 1.0%. Use 3.0% agar if rapid growers outgrow others.

(c) Goss et al. cultivated *B. chauvoei* and other organisms on a medium prepared as follows:
  (1) Grind 500.0 g. beef liver and add 1000.0 cc. water.
  (2) Cook (1) in flowing steam one hour.
  (3) Strain thru cheese cloth and cotton.
  (4) Add peptone (1.0%) and NaCl (0.5%).
  (5) Add 20.0 g. agar and heat in flowing steam until all is dissolved.
  (6) Adjust to pH = 8.2.
  (7) Clarify with egg albumin.
  (8) Filter and tube.
  (9) Autoclave at 15 pounds pressure for 20 minutes.

(d) Harvey prepared the medium as follows:
  (1) Prepare an infusion from 1000.0 g. liver (or other organ, placenta, etc.) and 1000.0 cc. water in the same manner as for ordinary infusion broth. (See variant (bb) medium 779.)
  (2) Dissolve 3.0% agar, 2.0% peptone and 1.0% NaCl in water.
  (3) Sterilize (2).
  (4) Mix equal parts sterile (1) and (3) while hot under aseptic conditions.
  (5) Tube in sterile tubes.

(e) Heller cultivated pathogenic anaerobes in the following medium:
  (1) Infuse one part beef liver with 4 parts distilled water in the refrigerator over night.
  (2) Bring to boil and strain thru cheese cloth.
  (3) Add 15.0 g. Peptone (Difco), 5.0 g. salt and 20.0 g. agar and dissolve by heating.
  (4) Titrate and adjust to pH = 7.2.
  (5) Cool to 60° and add white of egg and serum.
  (6) Autoclave for one hour.
  (7) Titrate and adjust to pH = 7.2.
  (8) Filter through cotton.
  (9) Tube.
  (10) Sterilize in the autoclave (time not given).

(f) Park, Williams and Krumwiede cultivated gonococci on the following medium:
  (1) Soak 5.0 pounds of finely chopped liver in 5000.0 cc. of tap water over night in an ice box or at room temperature.

(2) Weigh the kettle used in (1) and its contents.
(3) Heat at 45°C. for an hour.
(4) Boil for 30 minutes.
(5) Make up the loss by weight by the addition of water.
(6) Strain thru cheese cloth. Squeeze by twisting the cloth or use the meat press.
(7) If used at once dissolve 1.0% peptone and NaCl in (6).
(8) Dissolve 1.5% agar in (7) by heating in the autoclave at 10 to 15 pounds pressure for 30 minutes or by boiling over the free flame.
(9) If boiled over a free flame make up the loss in weight by the addition of water.
(10) Adjust the reaction to +0.2% to phenolphthalein.
(11) Cool to 50°C. and add one egg.
(12) Test the reaction and readjust if necessary.
(13) If more than 0.2% normal soda is required per liter, heat again for ten minutes.
(14) Filter thru cotton.
(15) Distribute in tubes or flasks.
(16) Sterilize at 15 pounds pressure for 30 minutes.

(g) Stafseth (Huddleson, Hesley and Torrey used the following medium for isolation and cultivation of *Bacterium abortus* (Bang):
(1) Grind fresh fat free beef liver in a meat chopper to a plastic mass.
(2) Mix (1) with 500.0 cc. of tap water.
(3) Place in flowing steam for 20 minutes.
(4) Remove the lid, and stir with a glass rod in order to mix thoroly.
(5) Continue the heating in flowing steam for 1¼ hours.
(6) Remove and filter thru a wire screen. (This infusion may be sterilized and stored until ready for use, or used at once.)
(7) Mix 500.0 cc. of (6) with 500.0 cc. of tap water, 20.0 g. washed agar, 10.0 g. Bacto peptone and 5.0 g. NaCl, and place in a covered container.
(8) Place in flowing steam for 30 minutes.
(9) Adjust to pH = 7.0.
(10) Add 1.0% dissolved egg albumin.
(11) Mix well and place in flowing steam for 90 minutes.
(12) Decant the liquid from the clot.
(13) Remove small clumps of albumin by filtering thru a discarded pressure cooker. Place glass wool, previously washed with dilute acid, in the barrel of the filter and a 30 mesh copper screen funnel for collecting large coagulated particles.
(14) Readjust to pH = 7.0 if necessary. The reaction should fall to pH 6.6 following sterilization.
(15) Sterilize at 15 pounds pressure for 30 minutes.
(16) For isolation work add sufficient quantity of a saturated aqueous solution of gentian violet so that the concentration be 1:10,000.

References: White (1917 p. 49), Bronfenbrenner and Schlesinger (1918 p. 125), (1918–19 p. 219), Heller (1921 p. 460), Goss, Barbarin and Haine (1921 p. 615), Harvey (1921–22 p. 69), Heller (1922 p. 9), Klimmer (1923 p. 201), Park, Williams and Krumwiede (1924 p. 131), Huddleson, Hasley and Torrey (1927 p. 356).

1670. Richardson's Mucosa Infusion Agar

Constituents:

1. Distilled water	500.0 cc.
2. Mucosa of hog intestine	250.0 cc.
3. Peptone	5.0 g.
4. NaCl	2.5 g.
5. Agar	3.8 g.

Preparation:
(1) Take small intestine of a hog in fresh condition without cutting, and wash thoroly with running water until the water runs perfectly clear.
(2) Lay the intestine open and scrape off the mucosa with a glass slide (150.0 g. of mucosa is easily obtained from one hog).
(3) To 250.0 cc. of the mucosa add 500.0 cc. of distilled water.
(4) Boil for 30 minutes. Cool.
(5) Boil again then neutralize and boil once more.
(6) Filter.

(7) Boil and add 5.0 g. peptone, 2.5 g. NaCl and neutralize again. Boil for 20 minutes.
(8) Filter. (The filtrate may be divided into two parts, and using half of the infusion as a liquid medium.)
(9) Add 1.5% agar to (8) and boil until solution is complete.
(10) Neutralize and cool to 68°C.
(11) Add a well beaten egg to (10) and boil until the egg is thoroly coagulated.
(12) Filter and tube.

Sterilization: Sterilize in the Arnold on each of three successive days for 30 minutes, and 30 minutes just before inoculation.

Use: Study of bodies found in carcinomatous tissue. Author was unable to obtain growth of carcinomatous bodies.

Reference: Richardson (1900–1 p. 73).

1671. Lichtenstein's Blood Clot Infusion Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Blood clots	500.0 g.
3. Peptone	
4. NaCl	
5. Agar	

Preparation:

(1) Run the blood clots thru a meat grinding machine.
(2) Mix (1) with a double amount of distilled water.
(3) Place in an enamel kettle and boil over a free flame for 10 minutes stirring constantly.
(4) Add concentrated acetic acid (amount not given).
(5) Boil for five minutes.
(6) Remove the flame and place the kettle in a slanted position so the precipitated material may settle out.
(7) Collect the clear liquid after it has cooled.
(8) Transfer into glass flasks and add the necessary amount (exact amount not given) of peptone, NaCl and agar.
(9) When the agar has dissolved, neutralize and place in the autoclave for 20 minutes.
(10) Filter.

Sterilization: Not specified.

Use: Cultivation of saphrophytic organisms. Author reported that diphtheria bacilli and streptococci grew on this medium better than on glycerol agar. Saphrophytic organisms requiring sugar in ordinary agar, grew luxuriantly on this medium.

Reference: Lichtenstein (1915–16 p. 362).

1672. Stuart's Stomach-Liver Infusion Agar

Constituents:

1. Water	1000.0 cc.
2. HCl	10.0 cc.
3. Liver	250.0 g.
4. Stomach, pig	200.0 g.
5. Peptone, Witte's (1.0%)	10.0 g.
6. Agar (1.2%)	12.0 g.

Preparation:

(1) Mix 250.0 g. of finely minced liver and 200.0 g. of that portion of a pig's stomach lining containing the gastric glands, in a liter of water containing 10.0 cc. of commercial HCl.
(2) Incubate the mixture for 18 hours in a water bath at 50°C.
(3) Make Biuret and tryptophane tests every six hours.
(4) Stop digestion by heating in the sterilizer in free flowing steam for 10 minutes.
(5) Allow to settle over night in the ice box and decant.
(6) Add 1.0% Witte's peptone and autoclave into solution.
(7) Add 1.2% agar.
(8) Adjust the reaction to pH = 7.0 and boil until the agar is dissolved.
(9) Adjust the reaction to pH = 7.2 to 7.4.
(10) Tube.

Sterilization: Method not given.

Use: To study variation in *Bacterium typhosum*.

Reference: Stuart (1924 p. 591).

1673. Bailey's Hormone Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Beef	500.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Agar	15.0 g.

Preparation:
- (1) Thoroly wash 15.0 g. of agar agar shreds in running water.
- (2) Dissolve (1) in a liter of distilled water and cool to between 50 and 60°C.
- (3) Add 500.0 g. of lean beef or beef heart, chopped to moderate fineness.
- (4) Bring to boil and cook slowly for 15 to 20 minutes.
- (5) Filter thru a 16 mesh sieve (cullander type) until clear.
- (6) Add 10.0 g. peptone and 5.0 g. NaCl.
- (7) Boil for 5 minutes.
- (8) Adjust to desired reaction (pH 7.5).
- (9) Allow to stand several minutes and decant the supernatant fluid.
- (10) Tube.

Sterilization: Sterilize by the fractional method or by autoclaving for about 20 minutes at 5 pounds pressure.

Use: Hormone medium.

Reference: Bailey (1925 p. 341).

1674. Fasiani and Zironi's Veal Autolysate Peptone Agar

Constituents:

1. Autolysate of veal liver	1000.0 cc.
2. Peptone (1.0%)	10.0 g.
3. NaCl (0.5%)	5.0 g.
4. Agar (2.0%)	20.0 g.

Preparation:
- (1) Dissolve 1.0% peptone 0.5% NaCl and 2.0% agar in an autolysate of veal liver.
- (2) Tube in 15 to 20.0 cc. lots.

Sterilization: Autoclave at 110°C. for 30 minutes.

Use: Isolation and cultivation of anaerobes.

Reference: Fasiani and Zironi (1918 p. 439). Taken from (1919 p. 147).

1675. Harvey's Basal Indicator Infusion Agar

See medium 1661 variant (v) for preparation of Infusion Agar. Add 1.0% of any desired carbohydrate, alcohol, etc., to the agar, adjust the reaction with brom thymol blue indicator to a pH value between 6.8 and 7.2. Add one of the following indicators:

(a)	Brom cresol purple	0.001%
(b)	Brom cresol purple	0.0005%
	Cresol red	0.0005%
(c)	China blue	0.0025%
(d)	China blue	0.005%
	Sodium rosolate	0.005%
(e)	China green	0.003%
(f)	China blue	0.0025 g.
	Phenol sulphonphthalein	0.001 g.

Keep the indicator in concentrated alcohol stock solution of such a strength that a definite amount can be measured out per liter of medium, e.g., 1.0 cc. of 1.6 per cent brom cresol purple per liter of medium.

1676. Jordan and Victorson's Lead Acetate Infusion Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone Witte's	30.0 g.
3. Beef, lean	1.0 lb.
4. Agar	15.0 g.
5. Lead acetate (10.0% soln.).	

Preparation:
- (1) Dissolve 2 in fresh meat broth (1 pound meat to 1 liter water, but exact procedure not given) by boiling.
- (2) Filter.
- (3) Dissolve agar in (2).
- (4) Adjust to 1.0% acid to phenolphthalein.
- (5) Tube.
- (6) Cool sterile (5) to 43°C., add 2 drops (0.1 cc.) of a 10.0% lead acetate solution prepared from recently sterilized water to each tube and mix well.

Sterilization: Sterilize (5) method not given.

Use: Differentiation of paratyphoid enteritidis group. Inoculate by sliding the needle in between the agar and wall of the tube. Authors reported that *B. paratyphosus B* and *B. enteritidis* produced H_2S, blackening the medium, while *B. paratyphosus A*, *B. suipestiver*, did not blacken the medium. Emile-Weil used a similar medium to determine H_2S production by *Bacillus leprae*.

Variants:
- (a) Emile-Weil prepared the medium as follows:
  - (1) Macerate 500.0 g. of finely divided beef with one liter distilled water for 12 hours.
  - (2) Boil slowly for 15 minutes.

(3) Allow to cool, skim and filter thru filter paper.
(4) Adjust the reaction so that the acidity is equal to 0.025 g. H_2SO_4 per liter (0.5%).
(5) Add 5.0 g. of agar and 10.0 g. Defresne peptone and heat for 15 minutes at 118°C.
(6) Filter and tube in 6 to 8.0 cc. lots.
(7) Heat at 112° for 15 minutes.
(8) When ready for use add 0.1 cc. of a 1 to 10 sterile solution of lead subacetate in distilled water to each tube.

(b) Tilley adjusted Jordan and Victorson's medium to pH = 7.2, sterilized the agar in the autoclave and added 3 drops of freshly prepared 10.0% lead acetate to each tube.

(c) Harvey added 1.0 drop (0.05 cc. to each tube (4.0 cc.) of agar (see variant (v) medium 1661) cooled to 45°C.

References: Jordan and Victorson (1917 p. 554), Emile-Weil (1917 p. 379), Tilley (1923 p. 115), Harvey (1921–22 p. 107).

1677. Elser and Huntoon's Basal Litmus Infusion Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Beef (lean)	500.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Agar agar	16.0 g.
6. Litmus, Kubel and Tiemann's	15.0 cc.

Preparation:

(1) Mix thoroughly 500.0 g. of chopped lean beef with 1000.0 cc. distilled water.
(2) Boil over a free flame for 15 minutes, stirring constantly.
(3) Filter.
(4) Add an emulsion of *Bacterium coli*.
(5) Incubate at 37°C. for 24 hours.
(6) Sterilize for 10 minutes.
(7) Filter until clear.
(8) Add 3, 4 and 5 that have previously been soaked in water, to (7).
(9) A portion of the filtrate is reinoculated with *Bacterium coli* to establish the sugar free condition of the product.
(10) Place (8) in a saturated salt solution bath and boil for 45 minutes, making provision for loss of water by evaporation.
(11) Add saturated Na_2CO_3 solution until slightly alkaline to litmus (allow for a slight augmentation of the acid during the subsequent steps).
(12) Cool to 55°C. and add the white of an egg and steam in Arnold sterilizer until clarification of the upper strata of the medium has occurred.
(13) Pipette off the clear supernatant fluid or filter.
(14) Transfer definite quantities of (13) to flasks.
(15) To 100.0 cc. of hot sterilized distilled water, add 10.0 g. of one of the added nutrients, and expose the solution to the action of live steam for 10 minutes. (Use old Jena glassware to avoid alkaline production.)
(16) Add to sterile (14) following the third sterilization, 1.5% of sterilized Kubel and Tiemann's litmus solution.
(17) Transfer to sterile tubes, slant and expose to incubator temperatures for several days.
(18) The final reaction of the medium should be very faintly alkaline to litmus.

Sterilization: Sterilize (14) by steaming for 15 minutes on each of three successve days.

Use: Cultivation of meningococci, pseudomeningococci and gonococci. The medium is suited primarily for stock cultures.

Added nutrients: The author added 1.0% of one of the following:

glucose
galactose
levulose
lactose
maltose
mannitol
dulcitol
inulin
dextrin
sucrose

Reference: Elser and Huntoon (1909 p. 406).

1678. Emile-Weil's Neutral Red Infusion Agar

Constituents:

1. Distilled water	1020.0 cc.
2. Beef	500.0 g.

3. Peptone (Defresne)	10.0 g.
4. Agar	5.0 g.
5. Neutral red	0.1 cc.

Preparation:

(1) Macerate 500.0 g. of finely divided beef with one liter distilled water for 12 hours.
(2) Boil slowly for 15 minutes.
(3) Allow to cool, skim and filter thru filter paper.
(4) Adjust the reaction to 1.5.
(5) Add 5.0 g. of agar and heat for 15 minutes at 118°C.
(6) Filter.
(7) Prepare a solution of 0.1 cc. neutral red in 20.0 cc. distilled water.
(8) Add 5.0 cc. of (7) to one liter of (6).
(9) Tube.

Sterilization: Sterilize at 112°C for 15 minutes.

Use: Cultivation of *Bacillus leprae*. Author reported that a change of color or fluorescence may be present or absent.

Variants: Harvey added 5.0 cc. of a 1.0% solution of neutral red and 5.0 g. of any desired carbohydrate, alcohol, etc., to medium 1661, variant (v).

References: Emile-Weil (1917 p. 379), Harvey (1921–22 p. 89).

1679. Lentz and Tietz's Malachite Green Infusion Agar

Constituents:

1. Loeffler's 2.0% meat infusion peptone agar.
2. Malachite Green.

Preparation:

(1) Add 1:1000, 1:2000, 1:4000, 1:6000, 1:8000 or 1:10,000 malachite green to Loeffler's 2.0% meat infusion peptone agar (method of preparation not given).
(2) Pour into petri dishes.

Sterilization: Not specified.

Use: Isolation colon-typhoid bacteria from feces. Author reported that coli grew weakly in 1:10,000 malachite green, typhoid well in 1:4000 and para B 1:2000.

Variants: Loeffler gave the following method of preparation:

(1) Prepare beef infusion 1 pound in two liters water.
(2) Add 30.0 g. agar.
(3) Add 7.5 cc. N/1 HCl.
(4) Boil 30 minutes.
(5) Add 7.0 cc. N/1 NaOH.
(6) Neutralize to litmus with sodium carbonate.
(7) Add 5.0 cc. N/1 sodium carbonate.
(8) Add nutrose to make 1% solution. (0.5% peptone may be used instead of nutrose).
(9) Boil.
(10) Distribute in 500.0 cc. flasks.
(11) Sterilize several hours on each of two successive days in streaming steam.
(12) Let cool slowly in sterilizer.
(13) Decant clear supernatant liquid.
(14) To each 100.0 cc. of agar add 2–2.5 cc. of a sterile 2.0% aqueous malachite green solution.
(15) Pour 15.0 to 20.0 cc. in each petri dish.
(16) Leave dishes open until agar is cool and hard.

References: Lentz and Tietz (1903 p. 2139), Loeffler (1906 p. 289).

1680. Zielleczky's Phenolphthalein Infusion Agar

Same as medium 783 but solidified by the addition of agar.

1681. Werbitzki's China Green Infusion Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Beef	500.0 g.
3. Peptone, Witte (1.0%)	10.0 g.
4. NaCl (0.5%)	5.0 g.
5. Agar (3.0%)	30.0 g.
6. China green (Boyer's)	

Preparation:

(1) Add 500.0 g. of finely chopped bone and sinew free lean beef to one liter of distilled water. Mix well.
(2) Weigh (1).
(3) Boil for 45 minutes stirring constantly.
(4) Weigh, and make up the loss in weight by the addition of distilled water.
(5) Filter thru a towel and measure the filtrate.
(6) Add 1.0% Witte's peptone and 0.5% NaCl to (5).
(7) Boil in the steamer.
(8) Cool in a cold water bath.

(9) Filter thru filter paper.
(10) Dissolve 3.0% agar in (9) in the steamer.
(11) Adjust the reaction by the addition of NaOH so that 1.3% NaOH will be required to bring the reaction to the neutral point of phenolphthalein.
(12) Heat.
(13) Filter in the steamer.
(14) Distribute in 100.0 cc. lots in Erlenmeyer flasks.
(15) Prepare a 0.2% china green solution.
(16) Add 1.4 to 1.5 cc. of (15) to each 100.0 cc. of sterile melted and cooled to 60–65°C. (14). Mix well.
(17) Pour into plates.

Sterilization: Method of sterilization of (14) not given.

Use: Isolation of typhoid bacilli from feces. Enrichment of typhoid bacilli. Smear the plates with the stool or feces suspension. Incubate 20 hours at 37°C. Wash the surface of the plates with 8 to 10.0 cc. physiological salt solution, and smear 1 to 3 loops of the salt solution suspension on Drigalski-Conradi medium.

Reference: Werbitzki (1909 p. 205).

1682. Teague's Victoria Blue Infusion Agar

Constituents:

1. Meat infusion	1000.0 cc.
2. Peptone, Witte (1.0%)	10.0 g.
3. NaCl (0.5%)	5.0 g.
4. Victoria blue 4R	

Preparation:

(1) Detailed method of preparation or composition of meat infusion agar not given except that it contains 1.0% Witte's peptone and 0.5% NaCl, and that it is to be prepared in the usual manner using the Arnold sterilizer.
(2) Clear (1) with egg white.
(3) Filter thru cotton.
(4) Titrate to +1.
(5) Add stock solutions (method of preparation not given) of Victoria blue 4R in varying amounts so that there is present 1/20, 1/30, 1/40 or 1/50 of dye, to sterile (4).

Sterilization: Sterilize by heating in Arnold for 3 successive days.

Use: Enrichment of *B. paratyphosus A* and *B. enteritidis*. Author reported that the dye inhibited growth of *B. paratyphoid B* but did not hinder growth of *B. paratyphoid A* or *B. enteritidis*.

Reference: Teague (1918 p. 1).

1683. Drennan and Teague's Crystal Violet Infusion Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Heart, beef	1.0 lb.
3. Peptone	10.0 g.
4. NaCl	2.5 g.
5. Agar	15.0 g.
6. N/1 NaOH	4.0 cc.
7. Crystal violet	1/70 g.

Preparation:

(1) Pass one pound of beef heart thru a meat chopping machine and soak in one liter of distilled water over night in an ice box.
(2) Squeeze the fluid thru a cheese cloth, heat to boiling and filter thru filter paper.
(3) Add 1.0% peptone, 0.25% NaCl, 1.5% agar-agar, 4.0 cc. of N/1 NaOH and heat for 30 minutes in the autoclave at 15 pounds pressure.
(4) Adjust the reaction to +1 (hot titration), clear with white of egg.
(5) Filter thru cotton.
(6) Distribute in flasks in 200.0 cc. lots.
(7) Add to the melted sterile agar 1/700% of crystal violet.
(8) Pour in plates.

Sterilization: Sterilize in the autoclave for 20 minutes at 15 pounds pressure.

Use: Isolation of *B. pestis* from lesions.

Reference: Drennan and Teague (1919 pp. 521–529).

1684. Meyer and Batchelder's Sulphite Gentian Violet Infusion Agar

Constituents:

1. Infusion agar	1000.0 cc.
2. Na_2SO_3 (10.0% soln.)	2.5 cc.
3. Gentian violet (1:1000)	25.0 cc.

Preparation:

(1) Prepare heart infusion agar using Berna peptone, according to Huntoon's method. (See medium 1863 for Huntoon's method.)
(2) Add 0.25% of a freshly prepared 10.0% solution) Na_2SO_3 and 1/400% (2.5 cc. of a 1:1000 solution per 100.0 cc.) of

gentian violet (improved Coleman Bell).

Sterilization: Sterilize in the autoclave.

Use: Isolation and cultivation of *B. pestis.*

Reference: Meyer and Batchelder (1926 p. 385).

1685. Müller's Indicator Infusion Agar

Same as medium 784 but solidified by the addition of 18.0 g. of agar per liter.

1687. Friedberger and Joachimoglus' Tellurite Placenta Infusion Agar

Constituents:

1. Placenta infusion agar.	100.0 cc.
2. Potassium tellurite (1.0%)	1.0 to 2.0 cc.

Preparation:

(1) Prepare neutral placenta infusion agar.

(2) Add from 1.0 to 2.0 cc. of a 1.0% potassium tellurite solution to each 100.0 cc. of (1).

Sterilization: Method not specified.

Use: Cultivation of *Bacillus typhi exanthematici* (*Bacillus proteus,* Weil and Felix). This organism produces an intense black color on this medium.

Reference: Friedberger and Joachimoglu (1918 p. 805).

1688. Ragit Agar

Constituents:

1. Water	1000.0 cc.
2. Ragit agar	42.0 g.

Preparation: (1) Dissolve 42.0 g. of Ragit agar in 1000.0 cc. of water. Ragit agar is a trade name of a dried medium marketed by Merck. It contains "Maggibouillon" Agar and Peptone in such amounts that 42.0 g. of powder in 1 liter of water gives a nutrient agar of the usual composition. (i.e. Beef infusion with 1.0% peptone and 2.0% agar.)

Sterilization: Not specified.

Use: General culture medium.

Variants:

(a) Tausz and Peter cultivated *Bacterium aliphaticum, Bacterium aliphaticum liquefaciens* and Paraffin bacteria, and prepared the medium as follows:

(1) Boil 42.0 g. Ragit agar with 1 liter of water for one hour.

(2) The reaction is slightly alkaline.

(3) Filter in the steamer.

(4) Sterilize on three successive days for 30 minutes in streaming steam.

(b) Müller dissolved 22.0 g. Ragit bouillon and 10.0 g. agar in 1000.0 cc. water to cultivate milk bacteria. The reaction was slightly alkaline.

References: Marx (1910 p. 361), Löhnis (1913 p. 17), Tausz and Peter (1919 p. 507), Müller (1917 p. 390).

1689. Bacto Nutrient Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Beef extract (Bacto)	3.0 g.
3. Peptone (Bacto)	5.0 g.
4. Agar (Bacto)	15.0 g.

Preparation:

(1) Dissolve 23.0 g. of Bacto Nutrient Agar (Dehydrated) in 1000.0 cc. distilled water by boiling or autoclaving.

(2) Restore loss if necessary.

(3) If sterilized 20 minutes at 15 pounds pressure pH = 6.6±.

Sterilization: Sterilize in the usual manner.

Use: General culture medium. The medium conforms to "Standard Methods" formula.

Reference: Digestive Ferments Co. (1925 p. 10).

1690. Bacto Nutrient Phosphate Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Beef extract (Bacto)	3.0 g.
3. Peptone (Bacto)	10 0 g.
4. Agar (Bacto)	15.0 g.
5. Na_2HPO_4	5.0 g.

Preparation:

(1) Dissolve 33.0 g. of Bacto Nutrient Phosphate Agar (Dehydrated) in 1000 0 cc. distilled water by boiling or autoclaving preferably by boiling.

(2) If sterilized 20 minutes at 15 pounds pressure pH = 7.5 ±.

(3) Cool to 50°C. before adding albuminous enrichment materials if these materials are to be added.

Sterilization: Sterilize in the usual manner.

Use: General culture medium.

Reference: Digestive Ferments Co. (1925 p. 11).

1691. Bacto Nutrient Agar 1.5% (Dehydrated)

Constituents:

1. Distilled water	
2. Beef extract (Bacto)	3.0 g.
3. Peptone (Bacto)	5.0 g.
4. Agar (Bacto)	15.0 g.
5. NaCl	8.0 g.

Preparation:

(1) Dissolve 31.0 g of Bacto Nutrient Agar 1.5% (Dehydrated) in 1000.0 cc. distilled water by boiling or autoclaving.

(2) Distribute as desired (Blood may be added in suitable proportions for blood agar).

(3) If sterilized at 15 pounds pressure for 20 minutes pH = 7.0 .

Sterilization: Sterilize in the usual manner.

Use: General culture medium.

Reference: Digestive Ferments Co. (1925 p. 11).

1692. Bacto Nitrate Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Beef extract (Bacto)	3.0 g.
3. Peptone (Bacto)	5.0 g.
4. KNO_3 (C. P.)	1.0 g.
5. Agar (Bacto)	12.0 g.

Preparation:

(1) Dissolve 21.0 g. Bacto Nitrate Agar (Dehydrated) in 1000.0 cc. of water by boiling or autoclaving, preferably the latter.

(2) Tube.

(3) If sterilized at 15 pounds for 20 minutes pH = 6.8±.

Sterilization: Sterilize in the usual manner.

Use: Study nitrate reduction.

Reference: Digestive Ferments Co. (1925 p. 13).

1693. Banning's Basal Meat Extract Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. Meat extract	1.0 g.
4. Agar	10.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Do not adjust the reaction.

(3) Dissolve one of the added nutrients in (2).

Sterilization: Do not heat over 75°C. when sterilizing (method not given).

Use: To study oxalic acid formation by acetic acid bacteria. Meier used a similar medium to study the bacterial count of milk and whey.

Added nutrients: The author added one of the following:

Methyl alcohol	10.0 g.
Ethyl alcohol	30.0 g.
Propyl alcohol	20.0 g.
Butyl alcohol	10.0 g.
Amyl alcohol	5.0 g.
Glycerol	10.0 g.
Ethylene glycocol	10.0 g.
Erythritol	10.0 g.
Mannitol	10.0 g.
Succinic acid	10.0 g.
Acetic acid	10.0 g.
Propionic acid	10.0 g.
Butyric acid	5.0 g.
Iso butyric acid	5.0 g.
Valearic acid	5.0 g.
Glyoxalic acid	2.5 g.
Lactic acid	10.0 g.
Malonic acid	2.5 g.
Pyrotaric acid	2.5 g.
Malic acid	10.0 g.
Salicylic acid	5.0 g.
Tartaric acid	10.0 g.
Potassium benzoate	5.0 g.
Glycocoll	10.0 g.
Leucine	5.0 g.
Tyrosine	2.5 g.
Potassium urate	5.0 g.
Creatin	2.5 g.
Creatinine	2.5 g.
Urea	10.0 g.
Hippuric acid	5.0 g.
Sarcosine (amount not given)	
Glucose	20.0 g.
Levulose	20.0 g.
Galactose	20.0 g.
Maltose	20.0 g.
Sucrose	20.0 g.
Lactose	20.0 g.
Raffinose	10.0 g.
Rhamnose	10.0 g.
Arabinose	20.0 g.
Starch (wheat)	10.0 g.
Inulin	10.0 g.
Glycogen	10.0 g.
Dextrin	10.0 g.
Gum arabic	10.0 g.

Variants: Meier studied the bacterial counts of milk and whey, using the following medium:

(1) Dissolve 15.0 g. of agar, 10.0 g. Liebig's meat extract, 10.0 g. Witte's peptone, 5.0 g. NaCl and 2.0% glycerol, 2.0% glucose or 4.5% lactose in 1000.0 cc. water.
(2) Neutralize to litmus and then add 10.0 cc. N/1 soda solution per liter medium.
(3) Sterilization not specified.

References: Banning (1902 p. 395 and 426), Meier (1918 p. 438).

1694. Heim's Meat Extract Salt Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	12.5 g.
3. Meat extract	10.0 g.
4. Peptone	10.0 g.
5. NaCl	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Neutralize with NaOH to litmus.
(3) Filter and distribute into culture plates containing different amounts of NaOH solution so that the final volume be 10.0 cc. 0.5% alkaline gave the best results.

Sterilization: Sterilize in steam (exact method not given) and allow plates to dry for 1 day with the cover removed.

Use: General culture medium. Heim used the medium to study effect of alkalinity and acidity on growth of anthrax bacillus.

Variants: The following authors prepared the medium as indicated:

(a) Ravenel (1899).
  (1) Add 10.0 g. peptone, 5.0 g. NaCl and 5.0 g. Liebig's beef extract to 500.0 cc. water.
  (2) Boil (1) for 3 minutes and neutralize (indicator not specified).
  (3) Chop 12.0 g. agar into small pieces and add to 500.0 cc. of water.
  (4) Place in the autoclave and run the pressure to two atmospheres (135.1°C.). Turn out the flame.
  (5) Allow the autoclave to cool to 100°C. before opening.
  (6) Cool (2) and (5) to 60°C. and mix.
  (7) Beat the whites of two eggs in 50.0 cc. water and add to (6). (Blood serum may be used instead of egg white.)
  (8) Boil for 3 to 5 minutes and filter thru paper.
  (9) Sterilization not specified.

(b) Frost (1903).
  (1) Add 15.0 g. agar to 500.0 cc. of water and dissolve by heating to 120°C. in the autoclave, shutting off the gas and allowing to cool, or boil until the agar is dissolved (about 30 minutes) and make up the loss of water due to evaporation.
  (2) Weigh out 3.0 g. of beef extract such as Liebig's and add 500.0 cc. of water.
  (3) Add 10.0 g. peptone and 5.0 g. NaCl to (2).
  (4) Heat until solution is complete.
  (5) Neutralize to phenolphthalein.
  (6) Cool to 60° and add one egg.
  (7) Add (6) to (1).
  (8) Boil until albumin is coagulated and floats in the clear liquid and restore the weight.
  (9) Readjust the reaction if necessary.
  (10) Add 0.5% normal HCl.
  (11) Filter thru cotton supported in a coil of wire using a suction pump.
  (12) Tube.
  (13) Sterilize in steam for 15 minutes on 3 successive days or in the autoclave for 20 minutes at 120°C. Slant some of the tubes after final sterilization.

(c) Hesse (1908).
  (1) Dissolve 10.0 g. Witte's peptone, 5.0 g. Liebig's meat extract and 8.5 g. NaCl in 500.0 cc. distilled water by boiling.
  (2) Dissolve 5.0 g. agar in 500.0 cc. distilled water and boil.
  (3) Filter (1) thru a filtering apparatus and (2) thru paper.
  (4) Mix the two filtrates.
  (5) Make up to 1 liter and boil again.
  (6) Distribute in 10.0 cc. lots in tubes.
  (7) Do not add alkali.
  (8) Sterilize in the autoclave under 2 atmospheres pressure for 20 minutes.

(d) Hesse (1908).
  (1) Dissolve 8.5 g. NaCl, 5.0 g. Liebig's

meat extract, 10.0 g. Witte's peptone and 5.0 g. agar in 1000.0 cc. water.
  - (2) Distribute in 10.0 cc. lots into test tubes that will not give up alkali by sterilization.
  - (3) Adjustment of reaction or sterilization not specified.
- (e) Stokes and Hachtel (1909).
  - (1) Dry agar at 105° for 30 minutes.
  - (2) Dissolve 4.5 g. of (1) in 500.0 cc. distilled water by boiling and make up loss in weight by adding distilled water.
  - (3) Dissolve 5.0 g. Liebig's beef extract, 10.0 g. Witte's peptone and 8.5 g. NaCl in 500.0 cc. distilled water.
  - (4) Mix (3) and (2) and boil 30 minutes.
  - (5) Make up the loss in weight by the addition of distilled water.
  - (6) Filter.
  - (7) Adjust the reaction to neutrality (indicator not specified).
  - (8) Tube in 9.0 cc. lots.
  - (9) Autoclave at 15 pounds pressure for 20 minutes.
- (f) Viehoever (1913) cultivated urea splitting organisms on the following medium:
  - (1) Dissolve 6.0 g. Witte's peptone, meat extract (amount not specified) 1.0 g. NaCl and 10.0 g. agar in 500.0 cc. water.
  - (2) Add 0.25% Na_2CO_3 to (1).
  - (3) Tube in 5.0 cc. quantities.
  - (4) Sterilize (method not given).
- (g) Bengis (1916).
  - (1) Dissolve 30.0 g. powdered agar in 1000.0 cc. distilled water.
  - (2) Add 10.0 g. Witte's peptone, 5.0 g. NaCl, and 5.0 g. meat extract to (1).
  - (3) Filter in incubating flasks.
  - (4) Sterilize in Bramhall-Dean autoclave.
- (h) Meier (1918) made bacterial counts of milk and whey using the following medium:
  - (1) Dissolve 15.0 g. agar, 10.0 g. Liebig's meat extract, 10.0 g. Witte's peptone and 5.0 g. NaCl in 1000.0 cc. distilled water.
  - (2) Neutralize to litmus and then add 10.0 cc. normal soda solution per liter of medium.
  - (3) Sterilization not specified.
- (i) Kligler and Defandorfer (1918).
  - (1) Dissolve 10.0 g. peptone, 3.0 g. beef extract and 5.0 g. NaCl in 1000.0 cc. water. (Meat infusion may be used instead of beef extract.)
  - (2) Add 15.0 g. agar to (1) and autoclave for 1 hour at 15 pounds pressure.
  - (3) Cool to 50°C., an egg white is added and steamed in Arnold 30 minutes.
  - (4) Adjust to pH = 7.4.
  - (5) Boil over free flame 6 or 7 minutes, filter, flask and autoclave (length of time not specified).
- (j) Hesse (Ball) (1919).
  - (1) Digest 5.0 g. agar in 500.0 cc. of water.
  - (2) Dissolve 10.0 g. peptone, 5.0 g. beef extract and 8.5 g. NaCl in 500.0 cc. water.
  - (3) Mix (1) and (2).
  - (4) Filter.
  - (5) Adjust the reaction to 1.0%.
  - (6) Tube.
  - (7) Sterilize in the autoclave.
- (k) Hesse (Tanner) (1919).
  - (1) Dissolve 5.0 g. agar in 500.0 cc. distilled water.
  - (2) Dissolve 10.0 g. peptone, 5.0 g. Liebig's beef extract, and 8.5 g. NaCl in 500.0 cc. distilled water.
  - (3) Mix (1) and (2).
  - (4) Filter.
  - (5) Tube.
  - (6) Sterilization not specified.
- (l) Malm (Besson) (1920).
  - (1) Dissolve 5.0 g. Liebig's meat extract (or Cibil's meat extract), 10.0 g. Chapoteaut's peptone, and 5.0 g. NaCl in 1000.0 cc. water.
  - (2) Soak 20.0 g. of chopped thread agar in cold water for several hours. Squeeze the water thru a cloth.
  - (3) Heat (2) and (1) at 100°C. until the agar is dissolved.
  - (4) Readjust the reaction if necessary.
  - (5) Allow to cool to 55 or 60°C.

(6) Beat the white of an egg in 100.0 cc. of water and add to (5).
(7) Mix well.
(8) Autoclave at 120°C. for one hour.
(9) Filter thru moistened Chardin filter using a hot water funnel.
(10) Tube.
(11) Sterilize at 115° for 20 minutes.

(m) Giltner (1921).
(1) Prepare an ordinary agar (method not given) from 5.0 g. NaCl, 5.0 g. Liebig's extract, 10.0 g. peptone and 30.0 g. agar in sufficient water to make 1000.0 cc.
(2) Adjust the reaction to 0 or +0.2%.
(3) Store in 100 0 cc. quantities in Erlenmeyer flasks.

(n) Wolf and Shunk (1921).
(1) Dissolve 10.0 or 20.0 g. agar, 3.0 g. Liebig's beef extract, 10.0 g. Peptone (Armour's) and 5.0 g. NaCl in 1000.0 cc. water by heating in the autoclave.
(2) Flask.
(3) Sterilize at 15 pounds for 15 minutes.
(4) Cool to 50°C.
(5) Pipette with sterile pipette, 10.0 cc. quantities into sterile test tubes and add appropriate quantities of strong acid or alkali to give desired reaction. (Authors used HCl sp. gr. 1.20 or 39.11% and NaOH sp. gr. 1.226 or approximately 20.0%.)
(6) Mix the tubes thoroly and cool. Do not sterilize.

(o) Giltner (1921).
(1) Place 500.0 g. of water in an agate water pail and add 15.0 g. of agar.
(2) Wash the agar well, separating the shreds and squeezing thru the hands. Wash until clean.
(3) Make up to the original volume the addition of tap water.
(4) Heat over a free flame until the agar is dissolved, stirring constantly.
(5) Add 3.0 g. of standard meat extract (or use 500.0 cc. meat infusion) to 500.0 cc. of tap water.
(6) Add 1.0% peptone and 0.5% NaCl.
(7) Cool (4) to 60°C. and mix with (6).
(8) Add 10.0 g. of albumin mixed with 100.0 cc. water.
(9) Autoclave at 15 pounds pressure for 2 hours.
(10) Adjust the medium to the desired reaction, by addition of normal NaOH or HCl.
(11) Filter while boiling hot thru plaited filter paper.
(12) Distribute as desired.
(13) Sterilize (method not given).

(p) Stitt (1923).
(1) Place 3.0 g. Liebig's meat extract, 10.0 g. peptone, Agar 2.0 or 3.0%, and 5.0 g. NaCl in 1000.0 cc. water in a rice cooker, or any other large straight sided granite-iron receptacle. The whites of one or two eggs may or may not be dissolved in the water.
(2) Place in the chamber of the dressing sterilizer and heat to between 5 and 10 pounds (110 to 115°) for at least 45 minutes.
(3) Adjust the pH as desired.
(4) Put back in the sterilizer for 30 minutes at the same temperature as in (2). The greater the pressure applied the darker the medium will be, so that a pressure not to exceed 3 pounds is used when a light medium is desired.
(5) Leave the medium in the sterilizer over night with the steam turned off.
(6) In the morning dump out the jelly mass and cut off and discard the bottom portion containing the sediment.
(7) Melt the remainder of the agar.
(8) Distribute in flasks (or nursing bottles).
(9) Sterilize (method not specified).
(10) Store until ready for use.

(q) Stitt (1923).
(1) Dissolve 15.0 g. of agar in 500.0 cc. of water in the inner compartment of a rice cooker.
(2) Cool to 55°C.
(3) Dissolve the whites of one or two eggs in 500.0 cc. of water.
(4) Prepare a paste from 3.0 g. Liebig's meat extract, 10.0 g. peptone and 5.0 g. NaCl by adding (3) little by little.
(5) Add the remainder of (3) to (4).
(6) Heat (5) to 50 to 55°C.

(7) Add (6) to (2).
(8) Adjust to a desired pH value by the addition of normal acid or alkali.
(9) Bring the agar to a boil in the inner compartment of a rice cooker.
(10) Filter in an autoclave or Arnold sterilizer thru filter paper wetted with boiling water. It may be filtered thru cotton or gauze if clearness of the medium is not an essential.
(11) Sterilization not specified.

(r) Stitt (1923).
(1) Prepare a paste of 3.0 g. meat extract, 10.0 g. peptone, 5.0 g. NaCl, 15.0 g. powdered agar and the white of one egg by mixing with a little water in a mortar.
(2) Add the remainder of the 1000.0 cc. of water and place in a rice cooker.
(3) Boil.
(4) Filter thru absorbent cotton placed between two layers of gauze in a hot funnel.
(5) Reaction is between +0.7 and +0.8.
(6) Sterilization not specified.

(s) Park, Williams and Krumwiede (1924).
(1) Dissolve, 2.0 to 5.0 g. Beef extract (Liebig's or Armour's), 10.0 g. peptone, and 5.0 g. NaCl in 1000.0 cc. water by boiling over a fire.
(2) Boil 15 minutes.
(3) Determine the reaction and adjust, if necessary, by the addition of normal NaOH.
(4) Dissolve 1.5% (or 2.0%) agar in (3) by heating in the autoclave at 10 to 15 pounds for 30 minutes or by boiling over the free flame.
(5) If boiled over a free flame make up the loss in weight by the addition of water.
(6) Adjust the reaction.
(7) Cool to 50°C. and add one egg.
(8) Heat in the autoclave at 10 to 15 pounds for 30 minutes, or in an Arnold sterilizer for one hour. Filter.
(9) Test the reaction and adjust if necessary.
(10) If more than 0.2% normal soda is required per liter, heat again for 10 minutes.
(11) Filter thru cotton.
(12) Distribute in tubes or flasks.
(13) Sterilize at 15 pounds pressure for 30 minutes.

References: Heim (1895 p. 193), Ravenel (1899 p. 605), (1899–1900 p. 89), Frost (1903 p. 16), Hesse (1908 p. 441), (1908 p. 89), Stokes and Hachtel (1909 p. 40), Viehoever (1913 p. 214), Bengis (1916 p. 392), Meier (1918 p. 435), Kligler and Defandorfer (1918 p. 438), Ball (1919 p. 80), Tanner (1919 p. 51), Besson (1921 p. 43), Giltner (1921 p. 386), Wolf and Shunk (1921 p. 325), Giltner (1921 p. 40), Heinemann (1922 p. 35), Stitt (1923 pp. 36, 37), Park, Williams and Krumwiede (1924 p. 117).

1695. Heinemann's Meat Extract Agar

Constituents:

1. Water (tap)	1000.0 cc.
2. Agar (1.5%)	15.0 g.
3. Beef extract	3.0 g.
4. Peptone, Witte (1.0%)	10.0 g.

Preparation:
(1) Weigh accurately a sauce pan without the lid.
(2) Measure 1000.0 cc. of tap water into the pan. Add 300.0 cc. of water to allow for evaporation. Heat over the gas.
(3) Add 15.0 g. of shredded agar to (2) and boil slowly until solution is complete.
(4) Add 3.0 g. of beef extract to (3).
(5) When dissolved remove from the flame and slowly dust in 10.0 g. of Witte's peptone. Stir constantly until dissolved.
(6) Adjust the reaction to alkaline to litmus or neutralize to phenolphthalein and then add 0.5% normal HCl.
(7) Allow the agar to cool to 60°C.
(8) Dissolve the whites of two eggs in 75.0 cc. of water, and stir well into (7).
(9) Heat, without stirring, on a piece of asbestos over the flame.
(10) Make up the weight due to the evaporation of water, or boil until the proper weight is obtained if the mixture weighs too much.

(11) Filter thru paper or absorbent cotton, using a vacuum pump.
(12) Tube.

Sterilization: Sterilize at 120°C. for 5 minutes.

Use: General culture medium.

Variants: The following methods of preparation have been described by authors indicated:

(a) Committee A. P. H. A. (1916).
  (1) Dissolve 3.0 g. Liebig's beef extract and 5.0 g. peptone in 1000.0 cc. water by boiling. Make up the loss in weight due to evaporation.
  (2) Filter hot thru paper.
  (3) Add 12.0 g. of oven dried agar, or 15.0 g. of market agar, and boil or heat in the autoclave to dissolve.
  (4) Restore the loss in weight due to evaporation.
  (5) If an autoclave is to be used the peptone and beef extract may be added to about 300.0 g. of water and the agar to 700.0 g. Autoclave for 15 minutes at 15 pounds pressure. Filter the hot broth thru filter paper and mix the filtrate with the agar. Filter thru absorbent cotton.
  (6) Adjust the reaction between +0.5 and +1.0.
  (7) Cool to 45° and heat to boiling for 15 minutes.
  (8) Filter thru paper or absorbent cotton until the medium is clear.
  (9) Tube in 10.0 cc. quantities or distribute in flasks.
  (10) Sterilize at 15 pounds pressure for 30 minutes. The medium may be sterilized on 3 successive days for 20 minutes after the agar has melted.

(b) Noyes (1916).
  (1) Dissolve 15.0 g. agar, 10.0 g. peptone and 5.0 g. Liebig's extract in 1000.0 cc. water.

(c) Committee A. P. H. A. (1917).
  (1) Add 3.0 g. of beef extract, 5.0 g. of peptone and 12.0 g. of agar, dried for 30 minutes at 105°C. before weighing to 100.0 cc. of water.
  (2) Boil over a water bath until all the agar is dissolved, and then make up the loss by evaporation.
  (3) Cool to 45°C. in a cold water bath, then warm to 65°C. in the same bath without stirring.
  (4) Make up the lost weight, titrate and if the reaction is not already between +0.5 and +1.0 adjust to +1.0.
  (5) Filter thru cloth and cotton until clear.
  (6) Distribute in 10.0 cc. lots in test tubes, or distribute in larger quantities if desired.
  (7) Sterilize in the autoclave at 15 pounds (120°C.) for 15 minutes after the pressure reaches 15 pounds.

(d) Committee S. A. B. (1918).
  (1) Prepare according to Committee A. P. H. A. (1916–1917). May be clarified with white of egg.
  (2) Adjust to pH = 6.6 to 7.4.
  (3) Sterilize.

(e) Tanner (1918).
  (1) Add 3.0 g. beef extract and 5.0 g. peptone to 1000.0 cc. distilled water.
  (2) Heat agar to 105°C. for 30 minutes.
  (3) Weigh out 12.0 g. of (2) and add to (1).
  (4) Boil over a water bath or cook in an autoclave until solution is complete.
  (5) Make up the loss due to evaporation.
  (6) Cool to 45 or 50°C. and add 10.0 g. of desiccated egg albumin per liter.
  (7) Heat in the autoclave for 30 minutes.
  (8) Make up the loss due to evaporation.
  (9) Adjust the reaction to +1.0.
  (10) Filter thru cotton or cloth until clear, or centrifuge in a Sharples centrifuge.
  (11) Tube.
  (12) Sterilize in the autoclave at 120°C.

(f) Dawson (1919).
  (1) Dissolve 10.0 g. peptone, 10.0 g. meat extract and 20.0 g. agar in 1000.0 cc. water.
  (2) Neutralize (indicator not given).
  (3) Sterilization not given.

(g) Committee A. P. H. A. (1920).
  (1) Same as for 1917, but adjust the reaction to a faint pink with

phenol red, or adjust to a +1.0 to phenolphthalein if the reaction is not between +0.5 and +1.0.

(h) Cohen (1922).
 (1) Dissolve 10.0 g. Difco peptone, 3.0 g. Liebig's beef extract and 20.0 g. of shredded agar in 1000.0 cc. of water.
 (2) Adjust to pH = 7.0.
 (3) Method of sterilization not specified.

(i) Committee A. P. H. A. (1923). Same as for 1917 (see variant (c) above, but adjust the reaction to a pH = 6.2 to 7.0.

(j) Park, Williams and Krumwiede (1924).
 (1) Soak 15.0 g. of agar (12.0 g. if dried in the oven at 105°C. for 30 minutes) in water and wash.
 (2) Dissolve 3.0 g. beef extract (Liebig's or equivalent) 5.0 g. Peptone (Armour, Difco, Fairchild's or equivalent) and (1) in 1000.0 cc. water.
 (3) Adjust the reaction to between +0.5 and +1.0 (phenolphthalein) or about pH 6.6 to 7.4, if necessary.
 (4) Tube.
 (5) Sterilize at 15 pounds for 15 minutes.
 (6) Cool rapidly.

(k) Park, Williams and Krumwiede (1924).
 (1) Dissolve 0.3% (3.0 g.) beef extract and 0.5% (5.0 g.) peptone in 400.0 cc. distilled water by boiling on the stove.
 (2) Adjust the reaction to between +0.5 and +1.0 (phenolphthalein) or about pH 6.6 to 7.4, if necessary.
 (3) Filter thru paper or paper pulp.
 (4) Soak 1.5% market agar (1.2% if oven dried) in water, and wash under tap in a sieve.
 (5) Add to (4) 600.0 cc. of distilled water minus the water absorbed during the washing. Weigh.
 (6) Mix (5) and (3).
 (7) Heat on the stove until the agar is completely melted, stirring constantly.
 (8) Boil and stir constantly for 20 minutes.
 (9) Make up the loss in weight by the addition of hot distilled water.
 (10) Readjust the reaction if necessary.
 (11) Filter thru cotton or paper pulp in a Buchner funnel, or run thru a Sharples centrifuge until clear.
 (12) Tube.
 (13) Sterilize in the autoclave for 20 minutes after the pressure reaches 15 pounds, or in streaming steam on 3 successive days for 20 minutes, after the agar is completely melted.

(l) Committee A. P. H. A. (1925).
 (1) Add 3.0 g. beef extract, 5.0 g. peptone and 15.0 g. agar (undried market product as stored in the ordinary laboratory cupboard) to 1000.0 cc. distilled water.
 (2) Boil until all the agar is dissolved.
 (3) Cool to 45°C. in a cold water bath, then warm to 65°C. in the same bath without stirring.
 (4) Make up the lost weight with hot distilled water and adjust the reaction so that the pH value, after final sterilization, will be between 6.2 and 7.0.
 (5) Bring to a boiling temperature, stirring frequently, restore the lost weight with hot distilled water and clarify.
 (6) Distribute in the desired containers.
 (7) Sterilize in the autoclave at 15 pounds (120°C.) for 15 minutes after the pressure has reached 15 pounds.

References: Heinemann (1905 p. 11), Committee A. P. H. A. (1916 p. 1316), Noyes (1916 p. 93), Committee A. P. H. A. (1917 p. 96), Committee S. A. B. (1918 p. 115), Tanner (1918 p. 48), Ball (1919 p. 77), Dawson (1919 p. 142), Committee A. P. H. A. (1920 p. 96), Levine (1921 p. 108), Cohen (1922 p. 189), Committee A. P. H. A. (1923 p. 4), Committee S. A. B. (1923 p. 9), Park, Williams and Krumwiede (1924 pp. 131, 132), Committee A. P. H. A. (1925 p. 98).

1696. Glaessner's Nährstoff Heyden Extract Agar

Constituents:

1. Water	1000.0 cc.

2. Agar	15.0 g.
3. Nährstoff Heyden	10.0 g.
4. NaCl	2.5 g.
5. Meat extract	0.5 g.

Preparation:

(1) Dissolve 15.0 g. of agar in 500.0 cc. water.
(2) Dissolve 3, 4 and 5 in 500.0 cc. water.
(3) Melt sterile (1) and cool to 44°C.
(4) Mix equal parts of sterile (3) and sterile (2) at 40°C.
(5) Pour in sterile plates.

Sterilization: Sterilize (1) and (2) separately (method not given).

Use: Cultivation of diphtheria bacilli.

Variants: The author used 1.0 g. meat extract and 5.0 g. NaCl instead of amounts indicated.

Reference: Glaessner (1900 p. 729).

1697. Guth's Selenicate Extract Agar

Same as medium 807, but solidified by the addition of 30.0 g. of agar.

1698. Conn and Breed's Nitrate Extract Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. Peptone	10.0 g.
4. KNO_3	1.0 g.
5. Beef extract	3.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Method not given.

Use: To determine the ability of bacteria to reduce nitrates.

Variants:

(a) Committee S. A. B. prepared beef extract agar according to the method of Committee A. P. H. A. (1920 see variant (g) medium 1695 and adjusted the reaction to pH = 6.6 to 7.4.
(b) Percival sterilized the medium on 3 successive days for 20 minutes each day.

References: Conn and Breed (1919 p. 278), Committee S. A. B. (1920 p. 128), Percival (1920 p. 163), Committee S. A. B. (1923 p. 10).

1699. Kotlar's Pancreas Peptone Agar

Constituents:

1. Water	1000.0 cc.
2. Powdered pancreas	50.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Agar	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Neutralize.

Sterilization: Method not given.

Use: Show effect of pancreas extract on growth of organisms. Author reported that the organisms were inhibited by the pancreas extract.

Reference: Kotlar (1895 p. 153).

1700. Bacto Andrade Maltose Agar (Dehydrated)

Constituents:

1. Water	
2. Beef extract (Bacto)	3.0 g.
3. Maltose (Bacto)	10.0 g.
4. Peptone (Bacto)	5.0 g.
5. Agar (Bacto)	12.0 g.
6. Andrade Indicator (Difco)	0.0275 g.
7. NaOH	

Preparation:

(1) Dissolve 30.0 g. of Bacto Andrade Maltose Agar (Dehydrated) in 1000.0 cc. of distilled water by boiling or autoclaving, preferably the latter.
(2) Tube.
(3) If sterilized 20 minutes at 15 pounds pH = 7.7±.

Sterilization: Sterilize in the usual manner, avoiding excess heat.

Use: General culture medium.

Reference: Digestive Ferments Co. (1925 p. 16).

1701. Bacto Andrade Mannite Agar (Dehydrated)

Same as 1700, but substituting Bacto Andrade Mannite Agar (Dehydrated) for Bacto Andrade Maltose Agar (Dehydrated).

1702. Bacto Andrade Dextrose Agar (Dehydrated)

Same as medium 1700, but using Bacto Andrade Dextrose Agar (Dehydrated) instead of Bacto Maltose Agar (Dehydrated).

1703. Bacto Andrade Lactose Agar (Dehydrated)

Same as medium 1700, but using Bacto Andrade Lactose Agar (Dehydrated) instead of Bacto Andrade Maltose Agar (Dehydrated).

1704. Bacto Andrade Saccharose Agar (Dehydrated)

Same as medium 1700, but using Bacto Andrade Saccharose Agar (Dehydrated) instead of Bacto Andrade Maltose Agar (Dehydrated).

1705. Percival's Basal Litmus Extract Agar

Constituents:

1. Water	1000.0 cc.
2. Meat extract, Lemco	5.0 g.
3. Peptone (Witte's)	10.0 g.
4. Agar	15.0 g.
5. Litmus	

Preparation:

(1) Soak 15.0 g. agar in 500.0 cc. water for 12 hours.
(2) Dissolve 2 and 3 in 500.0 cc. water.
(3) Mix (1) and (2) and boil or steam in the sterilizer for 20 to 30 minutes until solution is complete.
(4) Neutralize to phenolphthalein and then add 10.0 cc. of normal HCl per 1000.0 cc. of medium.
(5) Cool to 40 to 50°C. and add the white of an egg beaten up in a little water.
(6) Heat in the steam sterilizer for 90 minutes.
(7) Filter while hot thru a Chardin folded filter paper in a hot water funnel. If not clear, repeat the process.
(8) Add one of the added nutrients and sufficient litmus solution to give the desired color. (Chalk may be used instead of litmus.)

Sterilization: Steam for 20 minutes on 3 successive days.

Use: General culture medium.

Added nutrients: The author added 20.0 g. glucose or lactose.

Reference: Percival (1920 pp. 51, 57).

1706. Committee S. A. B. Lead Acetate Extract Agar

Constituents:

1. Water	1000.0 cc.
2. Beef extract	3.0 g.
3. Peptone	30.0 g.
4. Agar	15.0 g.
5. Lead acetate, basic	

Preparation:

(1) Prepare extract agar according to Committee A. P. H. A. (1916), see medium 1695.
(2) Adjust to pH = 7.2 to 7.6.
(3) Tube in 5.0 cc. quantities.
(4) Dissolve 1.0 g. of basic lead acetate in 1000.0 cc. water.
(5) Add 5.0 cc. of sterile (4) to each tube of sterile (3).
(6) Incubate to test sterility.

Sterilization: Sterilize (3) and (4) separately (method not given).

Use: Detection of production of hydrogen sulfid.

Reference: Committee S. A. B. (1922 p. 528).

1707. Conradi's Brilliant Green Picric Acid Extract Agar (Bezançon)

Constituents:

1. Water	900.0 cc.
2. Agar	30.0 g.
3. Meat extract, Liebig's	20.0 g.
4. Peptone, 10.0% soln	100.0 cc.
5. Brilliant green (1:1000)	10.0 cc.
6. Picric acid (1:100)	10.0 cc.

Preparation:

(1) Dissolve 2, 3 and 4 in 1. (Method not given.)
(2) Make alkaline (indicator not specified.
(3) To 1.5 liter of agar add 10.0 cc. of 1 to 1000 brilliant green and 10.0 cc. of a 1 to 100 picric acid solution.

Sterilization: Not specified.

Use: Differentiation of colon-typhoid group. Author reported that typhoid colonies were green and transparent. Paratyphoid colonies were large and the edges turned yellow; coli did not grow.

Variants:

(a) Harvey used Lemco extract instead of Liebig's extract.
(b) Stitt prepared the medium as follows:
  (1) Dissolve 20.0 g. of Liebig's extract and 30.0 g. of agar in 1000.0 cc. of water.
  (2) Adjust the reaction to +0.3 if necessary.
  (3) Filter thru cotton.
  (4) Distribute in 150.0 cc. lots in 250.0 cc. Erlenmeyer flasks.
  (5) Sterilize, method not given.

(6) Add 1.0 cc. of a 1 to 1000 aqueous solution of brilliant green and 1.0 cc. of a 1.0% solution of picric acid to each flask.
(7) Pour into large petri dishes.

References: Bezançon (1920 p. 345), Harvey (1921–22 p. 91), Stitt (1923 p. 49).

1708. Klinger's Malachite Green Extract Agar

Constituents:

1.	Water	2000.0 cc.
2.	Meat extract (Liebig's)	20.0 g.
3.	Peptone (Witte)	20.0 g.
4.	NaCl	10.0 g.
5.	Agar	80.0 g.
6.	Malachite green (Höchst 120)	

Preparation:

(1) Add 2, 3, 4 and 5 to 1.
(2) Heat for either 3 hours in streaming steam, or 2 hours at 110° or 1 hour at 120°C.
(3) Filter thru a thick layer of cotton.
(4) Remove 50.0 cc. of the agar and measure the rest.
(5) Allow the agar to solidify.
(6) After solidification add 0.05 g. Höchst 120 malachite green to each 100.0 cc. of agar.
(7) Liquefy the agar.
(8) Determine the reaction of the 50.0 cc. of agar removed in (4) and add sufficient normal NaOH to melted malachite green agar so that the alkalinity for 100.0 cc. of agar is 1.0 cc. of normal NaOH using phenolphthalein as an indicator.

Example: If 0.9 normal cc. are required to neutralize the 50.0 cc. agar, then 1.8 cc. would be required for 100.0 cc. If one wishes to adjust the reaction of 400.0 cc. of malachite green agar add 4 × 0.8 = 3.2 cc. normal NaOH.

(9) Pour into sterile plates.

Sterilization: Not specified.

Use: Isolation of typhoid bacilli. The author reported that other forms were inhibited.

Variants: Zipfel regenerated malachite green agar in the following manner:

(1) Remove the malachite green agar slants and plates from tubes and Petri dishes.
(2) Add 3.0% HCl to used malachite green agar slants or plates until the HCl covers the agar. Stir.
(3) Allow the acid to react for one hour, stirring continually.
(4) Pour the agar on a sieve.
(5) Wash the pieces of agar with a stream of water until the wash water is clear.
(6) Soak the agar in water for 24 hours, changing the water often.
(7) Place the agar on a sieve at the end of this time and allow to drip free from water.
(8) Liquify the agar particles in streaming steam.
(9) To a liter of (8) add 8.0 to 10.0 cc. of a 10.0% soda solution, 4.0 cc. of a filtered and sterilized solution of 20.0% peptone and 20.0% meat extract or meat equivalent.
(10) Sterilize for one hour in the steamer.
(11) The reaction of the agar determines the amount of malachite green to be added. There is an optimum for each batch of agar, and must be determined in each case.

References: Klinger (1906 p. 52), Zipfel (1917–18 p. 479).

1709. Köhler's Basal Agar

Constituents:

1. Nutrient agar.............. 1000.0 cc.

Preparation: (1) Dissolve one of the added nutrients in nutrient agar.

Sterilization: Not specified.

Use: To study the effect of acids and other materials on the growth of the typhoid bacillus. Other investigators used similar media for a variety of purposes.

Added nutrients: The author added 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0 45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0 95, 1.0, 1.5 or 1.1% of one of the following materials:

Lactic acid
Citric acid
H_3PO_4 (2.0%)
Tartaric acid
Alum
Carbolic acid
HCl (25%)
HNO_3 (30%)
H_2SO_4 (97%)
NaOH (33%)
KOH (33%)
Methyl violet
Fuchsin

Variants:

(a) Thoinot added any desired amount of

sterile lactic, tartaric or hydrochloric acid to sterile tubes of agar.

(b) Duval cultivated *B. leprae* in media prepared as follows:
  (1) Method of preparation or composiposition of 2.0% nutrient agar not given.
  (2) Adjust to 1.5% alkaline to phenolphthalein.
  (3) Distribute in 10.0 cc. lots.
  (4) Method of sterilization not given.
  (5) Prepare 2 0% solutions of cystine, (made from protein) leucine and tryptophane.
  (6) Sterilize (5) by passing thru a Berkefeld filter.
  (7) Add 5.0 cc. of one of (6) or in combination to melted (4).
  (8) Mix thoroughly and solidify in a slanted position.

(c) Abel mixed one part serum, ascitic fluid or hydrocele fluid, warmed, to 40 to 50°C. with one or two parts agar.

(d) Tanner added 1.0% of any desired carbohydrate, alcohol, etc. to plain agar.

(e) Dopter and Sacquépée added 2.0% of any desired carbohydrate, alcohol, etc., to nutrient agar.

References: Köhler (1893 p. 76), Thoinot and Masselin (1902 p. 35), Duval (1910 p. 655), Abel (1912 p. 26), Tanner (1919 p. 48), Dopter and Sacquépée (1921 p. 128).

1710. Wurtz's Nutrient Agar

Constituents:

1. Bouillon	1000.0 cc.
2. Agar	12.0 g.

Preparation:

(1) Dissolve 12.0 g. of agar in a liter of bouillon, slightly alkaline, by heating in a salt water bath.
(2) Cool to 50°C. and add the whites of two eggs beaten up in a liter of water.
(3) Heat for one minute at 125°C.
(4) Filter thru Chardin filter paper.
(5) Distribute in tubes.

Sterilization: Sterilize at 115°C. for 15 minutes.

Use: General culture medium.

Variants: The authors listed below have given the following method of preparation for similar media:

(a) Smith (1902).
  (1) Grind 5.0 g. of agar and boil for one or two hours in 100.0 cc. of water in a beaker. Add water from time to time.
  (2) Add 250.0 cc. of meat infusion (or extract) peptone solution.
  (3) Boil until the weight of the bouillon plus 5.0 g. of agar is obtained.
  (4) Cool to 60°C. or less and add one half the white of an egg dissolved in 25.0 cc. of boiled water.
  (5) Reboil for 10 or 15 minutes to coagulate the egg albumin.
  (6) Filter thru a folded and moistened paper.
  (7) Make up to the weight of the bouillon plus 5.0 g. agar.
  (8) If the agar is not clear, reheat and refilter.
  (9) Distribute.
  (10) Sterilize intermittently or in the autoclave.

(b) Roux and Rochaix (1911).
  (1) Dissolve 15.0 g. of agar in a liter of peptone bouillon by heating at 100°C.
  (2) Filter thru muslin.
  (3) Cool to 70–75°C. and add the white of an egg. Mix well.
  (4) Readjust the reaction if necessary.
  (5) Heat for 45 minutes.
  (6) Filter while hot.
  (7) Distribute in tubes.
  (8) Sterilize in the autoclave.

(c) Roux and Rochaix (1911).
  (1) Soak 25.0 g. of chopped agar for 24 hours in 500.0 cc. of water, acidulated by the addition of 6.0% HCl. Stir occasionally.
  (2) Wash thoroly with water.
  (3) Soak the agar for 24 hours in 500.0 cc. of a 5.0% ammonia solution.
  (4) Wash thoroly.
  (5) Place in a liter of bouillon and heat until dissolved.
  (6) Neutralize by the addition of a saturated solution of $NaHCO_3$.
  (7) Pass thru flannel and then filter using a hot water funnel.
  (8) Distribute into flasks or tubes.

(9) Sterilize at 115° to 120°C. for 30 minutes.

(d) Hoffmann (1912) made bacterial counts of the soil on a 0.1% peptone agar.

(e) Löhnis (1913).

(1) Cut agar into small pieces.
(2) Add 1.5% of (1) to bouillon.
(3) Allow to soak for one to two hours or overnight in a cool place.
(4) Heat in the autoclave until solution is complete at 1.5 atmospheres.
(5) Filter thru cotton wool.
(6) Sterilize in the autoclave.

(f) Roddy (1917).

(1) Add 1.0 to 3.0% agar to bouillon. (Shredded agar is used, cut into small pieces before adding.)
(2) Boil and stir until the agar is dissolved.
(3) Add water to make up the loss due to evaporation.
(4) Adjust the reaction.
(5) Filter in a warm place.
(6) Sterilize in the autoclave.

(g) Bezançon (1920).

(1) Wash 15.0 g. of finely chopped agar in water and soak for 12 hours.
(2) Add (1) to bouillon.
(3) Place in the autoclave at 120°C. for 15 minutes.
(4) Cool to 50°C.
(5) Adjust the reaction to slightly alkaline.
(6) Beat up the white of an egg in 50.0 cc. of water.
(7) Add (6) to (5).
(8) Autoclave at 115°C. for 20 minutes.
(9) Filter while hot.
(10) Tube.
(11) Sterilize in the autoclave at 115°C. for 10 minutes.

(h) Hilgermann and Weissenberg (1917–18) cultivated nematodes on the following medium. They reported that nematodes appeared first after 4 days or possibly after 10 or 14 days. Amoeba and bacteria grew at the end of 24 hours.

(1) Exact composition of nutrient bouillon not given.
(2) Adjust (1) so that it is alkaline.
(3) Mix 10.0 cc. of (2) and 90.0 cc. of water.
(4) Dissolve 1.5 g. agar in (3).
(5) Sterilize on three successive days for 30 minutes in a steamer.
(6) Pour in sterile petri dishes.
(7) Moisten the plant or material containing the nematodes and place on the solidified agar.

(i) Stitt (1923).

(1) Weigh 15.0 to 20.0 g. of powdered agar into a mortar.
(2) Make a paste by the addition of nutrient bouillon.
(3) When a smooth even mixture is made pour into the inner compartment of a rice cooker, and add the remainder of the 1000.0 cc. nutrient bouillon. Weigh the mixture.
(4) Fill the outer compartment of the rice cooker with 25.0% NaCl solution, and boil until the solution of agar is complete, (5 to 10 minutes boiling). Do not stir.
(5) Filter the agar thru a pledget of absorbent cotton, or cotton between two layers of gauze, in a funnel that has been heated with boiling water. The filter-stand with gauze cotton filter and flask may be placed in an Arnold sterilizer for 20 minutes and then filter the agar. (1.5% agar may be filtered thru paper.)
(6) Tube.
(7) Sterilize in the autoclave or Arnold.

(j) Park, Williams and Krumwiede (1924).

(1) Dissolve 1.5% agar in 1000 0 cc. bouillon by heating in the autoclave at 10 to 15 pounds for 20 minutes, or by boiling over a free flame.
(2) If boiled over a free flame make up the loss in weight by the addition of water.
(3) Adjust the reaction.
(4) Cool to 50°C. and add one egg.
(5) Heat in the autoclave at 10 to 15 pounds for 30 minutes or Arnold sterilizer for one hour. Filter.

(6) Test the reaction and adjust if necessary.
(7) If more than 0.2% normal soda is required per liter, heat again for 10 minutes.
(8) Filter thru cotton.
(9) Distribute in tubes or flasks.
(10) Sterilize at 15 pounds pressure for 30 minutes.

(k) Park, Williams and Krumwiede (1924) reported a very satisfactory medium can be made by simply using 0.5% or less of agar instead of the usual 1.5% employed. This can be diluted by the addition of $\frac{1}{3}$ of its bulk of an enrichment fluid. The finished medium should just "set" sufficiently for stab culture purposes. Nutrose (1.0%) may also be added.

(l) Park, Williams and Krumwiede (1924) gave the following medium as Musgrave and Clegg's Agar for the cultivation of amoeba:
(1) Mix 90.0% tap water, 10.0% ordinary nutrient broth (preparation not given) and 1.0% agar.
(2) Dissolve.
(3) Reaction neutral to phenolphthalein.
(4) Sterilize as usual (method not given).

(m) Cunningham (1924).
(1) Add 1.5% agar to bouillon.
(2) Steam for 30 minutes to dissolve the agar.
(3) Boil over an open flame for 15 minutes, stirring constantly.
(4) Adjust the reaction to a slight alkalinity using turmeric paper as an indicator (distinctly brown).
(5) Filter while hot thru a plug of cotton-wool in the bottom of an enamelled funnel.
(6) Tube.
(7) Sterilize intermittently in steam.

References: Wurtz (1897 p. 29), Smith (1902 p. 92), Roux and Rochaix (1911 pp. 115, 116), Hoffmann (1912 p. 387), Löhnis (1913 p. 16), Roddy (1917 p. 43), Bezançon (1920 p. 112), Hilgermann and Weissenberg (1917–18 p. 470), Klimmer (1923 p. 229), Stitt (1923 p. 36), Park, Williams and Krumwiede (1924 pp. 117, 118, 134), Cunningham (1924 p. 15).

1711. Warden's Salt Agar

Constituents:

1. Distilled water	1000.0	cc.
2. Bouillon	200.0	cc.
3. Agar	2.5	g.
4. Sodium bicarbonate	0.2	g.
5. $CaCl_2$	0.25	g.
6. KCl	0.45	g.
7. NaCl	10.8	g.

Preparation:
(1) Dissolve 3, 4, 5, 6 and 7 in 1 by aid of heat until fluid shows a translucent ground glass appearance.
(2) Add bouillon (composition or method of preparation not specified).
(3) Filter while hot, through gauze or cotton into test tubes or flasks.
(4) Adjustment of reaction not specified.

Sterilization: Sterilize once in autoclave (time not specified).

Use: Culture medium for gonococci. Author reported that when cool medium was semi-solid, and had a translucent silvery appearance. Nearly all strains of gonococci did not grow on this medium. If a few loopfuls of sterile human blood be placed on this surface, all strains grew.

Reference: Warden (1913 p. 94).

1712. Strössner's Regenerated Agar

Constituents:
1. Bouillon.
2. Agar (used).

Preparation:
(1) Place the used agar in an enamel container and melt in streaming steam (add no water).
(2) Measure the agar in a graduated cylinder.
(3) To each liter of agar add 40.0 g. of powdered charcoal and boil for 30 to 40 minutes in the steamer.
(4) Cool to 50°C. and add 40.0 cc. defibrinated blood per liter of agar.
(5) Boil in streaming steam once more for 40 minutes.
(6) Filter (method not given).
(7) Add 300.0 cc. meat infusion or 1 to 1.5% Liebig's bouillon to each liter of agar.

Sterilization: Method not given.

Use: Regenerated agar.

References: Ströszner (1917–18 p. 223), Zipfel (1917–18, p. 477).

1713. Besson's Basal Litmus Agar

Constituents:

1. Nutrient agar	1000.0 cc.
2. Litmus	

Preparation:

(1) Dissolve 2.0 to 4.0% of one of the added nutrients to plain agar.

(2) Just before use, melt (1) and add a sufficient quantity of litmus to give a light blue color.

Sterilization: Not specified.

Use: General culture medium.

Added nutrients: The author added 2.0 to 4.0% of any desired carbon source.

Variants: Bezançon gave the following method of preparation for a similar medium.

(1) Dissolve 1.0 g. of any desired carbohydrate in 20.0 cc. of litmus solution.

(2) Heat for 20 minutes at 105°C.

(3) Melt agar and cool to 30°C.

(4) Decant (2) and add 1.5 cc. of the litmus sugar solution to 5.0 cc. of agar.

(5) Mix well.

References: Besson (1920 p. 59), Bezançon (1920 p. 113).

1714. Mandelbaum's Basal Rosolic Acid Agar

Constituents:

1. Agar.
2. Rosolic acid (1.0% alcoholic solution).

Preparation:

(1) Dissolve an appropriate amount of one of the added nutrients in nutrient agar.

(2) To each 10.0 cc. of (2) add 0.3 cc. of a 1.0% solution of rosolic acid.

Sterilization: Not specified.

Use: To differentiate members of colon-typhoid group. The author used a similar medium to determine both acid production and hemolysis.

Added nutrients: The author added an appropriate amount of any desired carbohydrate, alcohol, etc.

Variants: The author added 2.0 cc. of human defibrinated blood to 5.0 cc. of nutrient agar (cooled to 48°C.) containing small amounts of glycerol, lactose or glucose.

Reference: Mandelbaum (1909 p. 2476).

1715. Zipfel's Regenerated Drigalski's Agar

Constituents:

1. Drigalski agar (used).
2. Peptone.
3. Meat extract.
4. Kahlbaum's litmus solution.

Preparation:

(1) Soak used agar slants and plates in a 3.0% HCl solution for one hour. Stir constantly.

(2) Wash the pieces of agar with water, and soak in water for 24 hours.

(3) Place in a 1.0% soda solution until the agar is completely blue.

(4) Wash with water, and allow the agar to drain.

(5) Melt the agar and add to 1 liter, 8.0 to 10.0 cc. of a 10.0% soda solution, 40.0 cc. of a filtered sterile 20.0% peptone solution and 20.0% meat extract or meat equivalent.

(6) Evaporate 250.0 cc. of Kahlbaum's litmus solution to 50.0 cc.

(7) Add 20.0 cc. of (6) to one liter of (5).

(8) The addition of lactose or other materials not specified.

Sterilization: Method not specified.

Use: Regenerated medium. This medium gave as good results as freshly prepared Drigalski agar.

Reference: Zipfel (1917–18 p. 478).

1716. Rothberger's Indicator Agar

Constituents:

1. Nutrient agar
2. Indicator (neutral red, etc.)

Preparation:

(1) Method of preparation of nutrient agar not specified.

(2) Prepare a saturated solution of neutral red.

(3) To each 10.0 cc. of melted sterile (1) add 3 or 4 drops of sterile (2).

Sterilization: Method of sterilization not given.

Use: Differentiation between typhoid and colon bacilli.

Variants:

(a) The author used 3 or 4 drops of a saturated watery solution per 10.0 cc. of agar of one of the following indicators instead of neutral red.
   (a) Saffranin.
   (b) Methylene blue.

(c) Water blue.
(d) Berlin blue.
(e) Gentian violet.
(f) Methyl violet.
(g) Crystal violet.
(h) Fuchsin.
(i) Methyl green.
(j) Malachite green.
(k) Iodine green.
(l) Guignets-green.
(m) Acid violet.
(n) Acid fuchsin.
(o) Phlexin red.
(p) Magdali red.
(q) Erythrosin.
(r) Benzoazurin.
(s) Benzopurpurin.
(t) Indulin.
(u) Nigrosin.
(v) Aniline blue.
(w) Corallin.
(x) Noggerath's color mixture.
(y) Chrysoidin.
(z) Ruby S.
(aa) Kongo red.
(bb) Dahlia.
(cc) Victoria blue.
(dd) Indigo carmine.
(ee) Orseille-extract.
(ff) Toluidin.
(gg) Magenta red.
(hh) Bismarck Brown.

(b) Hunter added 0.1 to 0.5 cc. of a concentrated watery solution of neutral red to nutrient agar.

References: Rothberger (1898 pp. 515, 517), (1899 p. 72), Hunter (1901 p. 614).

1717. Omelianski's Indicator Agar

Constituents:
1. Nutrient agar.............. 1000.0 cc.
2. Methylene blue (1.0% solution)

Preparation: (1) Add 10 drops of a 1:100 solution of methylene blue to agar.

Sterilization: Not specified.

Use: Differentiation of *Bact. coli* and *Bac. typhi*. Author reported that coli gives decolorization after 6–7 hours. Typhoid colonies show no decolorization until later. (See variant.)

Variants: The author added 0.1 cc. of a sterile 2.0% indigo-carmine solution to 10.0 cc. of sterile melted (not hot) agar. The medium was used for the differentiation of *Bact. coli* and *Bact. typhi*, *Bac. diphtheriae* and *Bac. Pseudodiphtheriae*. The author reported that coli organisms changed the blue agar to yellow after 15 to 20 hours. Typhoid organisms decolorized the agar after 3 or 4 days. *Bac. pseudodiphtheriae* grew very luxuriantly with yellow colonies, and after 2–3 days caused a decolorization nearly as intense as *Bact. coli*. *Bac. diphtheriae* grew less abundantly and did not cause decolorization.

Reference: Omelianski (1903 p. 4).

1718. Burnet and Weissenbach's Lead Acetate Agar

Constituents:
1. Nutrient agar.
2. Lead acetate.

Preparation:
(1) Preparation of agar not given.
(2) Prepare a 1:10 solution of neutral lead acetate in water.
(3) Add under aseptic conditions one drop of freshly prepared sterile (2) to each tube containing 4.0 cc. of melted sterile (1).
(4) Mix well.
(5) Slant.

Sterilization: Sterilize (1) and (2) separately; method not given.

Use: H_2S production. Author reported that the medium is darkened if H_2S is formed. Paratyphoid B and typhoid bacillus formed H_2S. Paratyphoid A did not produce H_2S.

Variants: Thompson prepared a similar medium as follows:
(1) Exact composition of Standard 1.5% agar not specified but it is to be prepared using "Difco" peptone.
(2) Adjust to pH 6.8 or 7.0.
(3) Distribute in 100.0 cc. lots in flasks. Method of sterilization not given.
(4) Prepare a 2.0% aqueous solution of Merck's subacetate. Store in a tightly stoppered bottle. This solution may be kept several weeks under these conditions.
(5) Just before use melt (3) and add to each 100.0 cc. lot, 20.0 cc. of (4).
(6) Distribute into sterile tubes, inoculate and pour into sterile plates.

References: Burnet and Weissenbach (1915 p. 567), Thompson (1920–21 p. 384).

1719. Noeggerath's Indicator Agar (Besson)

Constituents:

1. Agar	
2. Methylene blue (sat. soln.)	2.0 cc.
3. Gentian violet (sat. soln.)	4.0 cc.
4. Methyl green (sat. soln.)	1.0 cc.
5. Chrysoidine (sat. soln.)	4.0 cc.
6. Fuchsin (sat. soln.)	3.0 cc.

Preparation:

(1) Prepare saturated watery solutions of methylene blue, gentian violet, methyl green, chrysoidine and fuchsin.
(2) Mix 2.0 cc. of methylene blue, 4.0 cc. gentian violet, 1.0 cc. methyl green, 4.0 cc. of chrysoidine and 3.0 cc. of fuchsin.
(3) Add 200.0 cc. of distilled water to (2) and allow to stand several hours. The color should be greenish blue. If not, obtain the original color by adding either blue, green or red dye.
(4) Add 7 to 10 drops of sterile (3) to a tube of sterile melted nutrient agar.

Sterilization: Sterilize (3) at 100°C.

Use: General culture medium.

Reference: Besson (1920 p. 60).

1720. Gasser's Fuchsin Agar

Constituents:

1. Nutrient agar.
2. Fuchsin.

Preparation:

(1) Add twenty drops of a saturated aqueous solution of fuchsin to a tube of nutrient agar.
(2) Pour sterile (1) in petri dishes.

Sterilization: Method not given.

Use: Differentiation of colon-typhoid group. Author reported the typhoid culture was red after 24 hours at 37°C. while *B. coli communis* decolorized the medium.

Reference: Gasser (1890 p. 463).

1721. Krumwiede and Pratt's Dahlia Agar

Constituents:

1. Agar.
2. Dahlia.

Preparation: (1) Add dahlia in the ratio of 1 to 100,000 (0.5 cc. of 1.0% dahlia per 500.0 cc. agar) to nutrient agar.

Sterilization: Not specified.

Use: Detection of cholera vibrio. Author reported that cholera vibrio colonies were colored violet.

Reference: Krumwiede and Pratt (1913 p. 562).

1722. Meier's Glucose Infusion Agar

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.
3. Agar	15.0 g.
4. Peptone	10.0 g.
5. Glucose	5.0 g.

Preparation:

(1) Boil 500.0 g. of fat and tendon free beef in 1 liter of water.
(2) Filter.
(3) Dissolve 3, 4 and 5 in (2).
(4) Neutralize by the addition of KOH. Add KOH until turmeric paper is turned quite weakly brownish red.

Sterilization: Not specified.

Use: Bacterial count of milk. Goss, Barbarin and Haines cultivated *B. chauvoei* on a similarly prepared medium.

Variants: Goss, Barbarin and Haines prepared a similar medium as follows:

(1) Prepare beef infusion from 500.0 g. beef and 1000.0 cc. water. (Exact method not given.)
(2) Add 10.0 g. peptone, 5.0 g. NaCl and 20.0 g. Dextrose to (1).
(3) Adjust to pH = 8.2.
(4) Add 2% agar.
(5) Heat in flowing steam until agar is dissolved.
(6) Clarify with egg albumin.
(7) Filter and tube.
(8) Autoclave at 15 pounds pressure for 20 minutes.

References: Meier (1918 p. 436), Goss, Barbarin and Haines (1921 p. 615).

1723. Hitchens' Glucose Infusion Agar

Constituents:

1. Distilled water	2000.0 cc.
2. Beef, lean	1000.0 g.
3. Peptone	40.0 g.
4. KNO_3	4.0 g.
5. Glucose	4.0 g.
6. Agar	60.0 g.

Preparation:

(1) Stir 1000.0 g. of lean beef into 1000.0 g. of water.
(2) Incubate at 37°C. for 48 hours.

(3) Strain and heat in water bath to boiling and strain again.
(4) Dissolve 3 and 4 in (3).
(5) Adjust to pH = 7.5.
(6) Filter and autoclave at 15 pounds pressure for 30 minutes.
(7) Dry agar thoroly, weigh and wash in running water over night.
(8) Dissolve 60.0 g. (7) in 1000.0 cc. of water in the autoclave.
(9) Adjust to pH = 7.5 and clear by straining through cotton and gauze.
(10) Add glucose to (6) just before addition of agar solution.
(11) In making the mixtures the amount of hot fluid, 6.0% agar, necessary to obtain the various percentages, is diluted with hot distilled water to a volume equal to that of the double strength broth (9) also hot, and mixed.
(12) Check reaction (pH = 7.5).

Sterilization: Sterilize in autoclave at 15 pounds for 20 minutes.

Use: To study growth at different agar concentrations. The author recommended a 0.1% agar concentration for growth of aerobic and anaerobic bacteria.

Reference: Hitchens (1921 p. 391).

1724. Jackson and Muer's Liver Infusion Agar

Constituents:

1.	Water	1000.0 cc.
2.	Liver, beef	500.0 g.
3.	Agar	5.0 g.
4.	Peptone (Witte's)	10.0 g.
5.	Glucose	10.0 g.
6.	K_2HPO_4	1.0 g.

Preparation:

(1) Chop 500.0 g. beef liver into small pieces, add 500.0 cc. distilled water and boil slowly for two hours, stirring occasionally.
(2) Add 3 (dried at 105°C. for 30 minutes) to 500.0 cc. distilled water and digest for 30 minutes in an autoclave at 120°C. (15 pounds).
(3) After making up the loss by evaporation, strain the liver infusion thru a wire strainer, add 500.0 cc. filtrate to the agar solution.
(4) To the filtrate add 4 and 5, then (2). Weigh the infusion and container.
(5) After warming this mixture in a double boiler and stirring it for a few minutes to dissolve ingredients, titrate with N/20 sodium hydrate, using phenolphthalein as an indicator, and neutralize with normal sodium hydrate.
(6) Boil vigorously for 30 minutes in a double boiler, and 5 minutes over a free flame with constant stirring to prevent the caramelization of the dextrose.
(7) Make up any loss in weight by evaporation and filter thru cotton flannel and filter paper.
(8) Tube.

Sterilization: Sterilize in an autoclave for 15 minutes.

Use: Cultivation of *B. sporogenes* and other bacteria. Medium also used for presumptive test for *B. coli* in water analysis.

Variants: Harvey solidified medium 833 variant (a) by the addition of agar. He reported that when 1.0 acid, *B. bifidus* grew on this medium. When 4.0% acid *B. acidophilus* grew on this medium.

References: Jackson and Muer (1911 p. 290), (1911 p. 929), Harvey (1921–22 p. 110).

1725. Hall's Testicular Infusion Agar

Constituents:

1.	Distilled water	1000.0 cc.
2.	Testicles, beef	500.0 g.
3.	Peptone (Witte or Difco)	20.0 g.
4.	Agar	30.0 g.
5.	Glucose	5.0 g.
6.	NaH_2PO_4	3.0 g.

Preparation:

(1) Soak over night, 500.0 g. of ground beef testicles from which the tunica vaginalis has been stripped, in 1000.0 cc. distilled water at room temperature.
(2) Heat to 50°C. Keep warm in 37° incubator for one hour.
(3) Boil—strain, and restore to 1000.0 cc. If in excess do not boil to reduce volume,—overheating is injurious.
(4) Add 3, 4, 5 and 6 to (3).
(5) Soak at least an hour to soften the agar.
(6) Melt in autoclave at 10 pounds for 30 minutes.

(7) Titrate with N/1 NaOH to neutral point with phenolphthalein.
(8) Check the titre by repetition using N/20 NaOH—5.0 cc. should require 1.0 to 2.0 cc. to display color while hot.
(9) Tube.

Sterilization: Sterilize in autoclave at 10 pounds for 30 minutes.

Use: Cultivation of gonococci. The author reported that media with 1.0%, 2.0%, 3.0% or 4.0% agar were also tried with and without glucose and varying amounts of NaOH. The concentrations given were found to be the most satisfactory.

(a) Clark prepared the medium as follows:
  (1) Remove and discard tunica vaginalis from fresh beef testicles and rinse in running water and grind.
  (2) Mix ground testicles (500.0 g.) with equal weight of distilled water (500.0 cc.) at room temperature, and infuse over night.
  (3) In the morning heat to 50°C. for one hour. Heat in steam kettle or double boiler, stirring, until the proteins are coagulated and tend to collect in large flocculi. Do not heat over free flame.
  (4) Strain thru coarse cloth and, if necessary, add distilled water to 750.0 cc.
  (5) Dissolve 20.0 g. Peptone (Parke, Davis) and 3.0 g. $NaH_2PO_4 + H_2O$ in warm (4), (40°C.).
  (6) Adjust while warm to pH = 7.4 to 7.8. Heat to boiling and adjust more precisely.
  (7) Soak 25.0 to 30.0 g. agar in 250.0 cc. distilled water, and autoclave long enough to dissolve agar.
  (8) Add melted (7) to (6).
  (9) Add 5.0 g. glucose.
  (10) Mix thoroly and distribute into desired containers.
  (11) Autoclave at 15 pounds for 20 minutes.
  (12) Rotate tubes to mix before slanting.

(b) Harvey gave the following method of preparation:
  (1) Remove the tunica vaginalis of bulls testicles.
  (2) Mince finely.
  (3) Allow to macerate in water overnight.
  (4) Heat in a water bath with constant stirring until the proteins have been coagulated.
  (5) Filter thru coarse cloth.
  (6) Make up to volume, 750.0 cc., with hot distilled water.
  (7) Add to the fluid, while hot, 20.0 g. peptone and 3.0 g. dihydrogen sodium phosphate.
  (8) Allow the temperature to fall to 40°C.
  (9) Estimate and adjust the reaction to pH 7.6.
  (10) Add 30.0 g. agar previously dissolved in 250.0 cc. distilled water and finally 5.0 g. glucose.
  (11) Mix.
  (12) Distribute in test tubes.
  (13) Sterilize at 120°C.
  (14) Rotate the tubes to mix before allowing to solidify.

References: Hall (1916 p. 351), Clark (1920 p. 100), Harvey (1921–22 p. 98).

1726. Kligler's Lead Acetate Infusion Agar

Constituents:

1. Meat infusion agar (agar 7.5 to 10.0 g.)	1000.0 cc.
2. Glucose	0.1%
3. Lead acetate	0.05%

Preparation:
(1) Prepare meat infusion agar in the usual way. Use 0.75 to 1.0% agar, however. Details of method not given.
(2) The reaction should be between pH = 7.2 to 7.6.
(3) Dissolve the 0.1% glucose and 0.05% lead acetate in water separately.
(4) Cool sterile melted agar to 60° and add the sterile solution of glucose and lead acetate to the agar under aseptic conditions.
(5) Mix tubes thoroly.
(6) Incubate to test sterility.

Sterilization: Sterilize (2) and (3) separately—method not given.

Use: Differentiation of typhoid and paratyphoid group. Author reported that the stab cultures showed the following reactions:

B. typhi browned the medium along the line of growth.

B. paratyphi B produced browning and gas.

B. paratyphi A gave gas but no browning.

B. dysenteriae gave neither gas nor browning.

Variants: Harvey added 4 drops of sterile 30% glucose solution at 45°C. and 2 drops of a sterile 5.0% lead subacetate at 45°C. to 10.0 cc. of melted sterile infusion agar (see variant (v) 1661).

Reference: Kligler (1917 p. 1043), Harvey (1921–22 p. 107).

1727. Gage and Phelps' Neutral Red Infusion Agar

Constituents:

1. Meat infusion	1000.0 cc.
2. Agar	10.0 g.
3. Peptone (Witte)	10.0 g.
4. Glucose	3.0 g.
5. Neutral red (1.0% watery solution)	10.0 cc.

Preparation:

(1) Prepare 1000.0 cc. of meat infusion.
(2) Dissolve 2, 3 and 4 in (1).
(3) Add 10.0 cc. of a 1.0% watery solution of neutral red to (2).

Sterilization: Not specified.

Use: To study the neutral red reaction by members of the colon group. Color changes from red to yellow if the reaction is positive.

Reference: Gage and Phelps (1902 p. 408).

1728. Harvey's Malachite Green Infusion Agar

Add 10.0 g. glucose and from 16 to 25.0 cc. of a 1–1000 malachite green solution to 1000.0 cc. infusion agar, (see variant v medium 1661) with a reaction of 0.3% acid to phenolphthalein.

1729. Endo's Fuchsin Sulphite Infusion Agar

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Agar	30.0 g.
6. Lactose (C. P.)	10.0 g.
7. Fuchsin (alcoholic solution)	5.0 cc.
8. Sodium sulfite (10.0% soln.).	25.0 cc.

Preparation:

(1) Add 2, 3, 4 and 5 to 1.
(2) Boil well (time not given).
(3) Filter and neutralize.
(4) Add 10.0 cc. of 10.0% soda solution to make alkaline.
(5) Add 10.0 g. of chemically pure lactose and 5.0 cc. of a filtered alcoholic fuchsin solution (strength not given).
(6) Add 10.0 cc. of a 10.0% sodium sulfite solution. This nearly decolorizes the red medium. The sulfite solution must be kept in a well stoppered bottle or prepared fresh.
(7) Distribute in 15.0 cc. lots.
(8) Store sterile (7) in a dark place until ready for use.

Sterilization: Sterilize for 30 minutes in a steaming apparatus.

Use: Diagnosis of typhoid fevers. Author reported that typhoid colonies were colorless; coli colonies red, and after 24 hours a green shiny fuchsin crystal-like upper surface appeared on them.

Variants: The following authors have prepared the medium as indicated below:

(a) Reitz.
  (1) Boil 500.0 g. of finely chopped beef with 1 liter water, 10.0 g. Witte's siccum peptone and 30.0 g. agar.
  (2) Filter and neutralize.
  (3) Make alkaline by adding 10.0 cc. of a 10.0% soda solution.
  (4) Add 10.0 g. of c. p. lactose and 5.0 cc. of a filtered alcoholic fuchsin solution.
  (5) Decolorize the red medium by the addition of 25.0 cc. of a freshly prepared 10.0% Na_2SO_3 solution.
  (6) Sterilization not specified.

(b) Strószner prepared a regenerated Endo agar as follows:
  (1) Place the Endo agar in enamel containers and melt in streaming steam.
  (2) Measure the melted agar in a graduated cylinder.
  (3) To a liter of (2) add 50.0 g. of powdered charcoal and boil for 30 to 40 minutes in a steamer (Stir).
  (4) Cool to 50°C. and add 50.0 cc. defibrinated blood per liter of agar at room temperature. Add

blood slowly and stir to avoid the formation of clumps.

(5) Boil in the steamer for 40 minutes.

(6) Filter (method not given).

(7) Sterilize (method not given). (The agar is light red.)

(8) To a liter of agar add 300.0 cc. of infusion broth or 1.0 to 1.5% Liebig's extract broth, 4.0 g. lactose, 4.0 cc. alcoholic concentrated fuchsin solution and 2.0 cc. of a 10% Na_2SO_3 solution. (Alkali need not be added.)

(c) Zipfel prepared regenerated Endo agar as follows:

(1) Remove the Endo agar slants and plates from tubes and petri dishes.

(2) Add 3 0% HCl to used Endo agar slants or plates until the HCl covers the agar. Stir.

(3) Allow the acid to react for one hour, stirring continuously.

(4) Pour the red agar on a sieve. The HCl solution may be saved and utilized (see end).

(5) Wash the pieces of agar with a stream of water until the wash water is clear.

(6) Soak the agar in water for 24 hours, changing the water frequently.

(7) Place the agar on a sieve at the end of this time and allow to drip free from water.

(8) Liquify the agar particles in streaming steam.

(9) To a liter of (8) add 8 to 10.0 cc. of a 10% soda solution, 4.0 cc. of a filtered and sterilized solution of 20.0% peptone and 20.0% meat extract or meat equivalent.

(10) Sterilize for one hour in the steamer.

(11) To prepare Endo agar add 3.0 cc. of a concentrated fuchsin solution, 25.0 cc. of a 10.0% Na_2SO_3 solution freshly prepared and 8.0 g. lactose dissolved in a little water, per liter of (10).

(12) Pour into sterile plates.

(13) To utilize the HCl washings from (4), boil and then neutralize with soda, filter (charcoal may be added) and concentrate by evaporation. The washings may be used directly in the preparation of agar after neutralization and filtration by adding 2 or 3% peptone per liter.

(14) To prepare plain agar from used Endo agar take the slants and plates and treat with a 0.5% NaOH solution after washing (5) until the red color disappears. Wash in water until the agar reacts neutral. Add peptone and meat extract as in (9). To completely remove the pink color add 1.0 cc. of a 1.0% bismark brown solution per liter of agar.

(d) Levine gave the following method of preparation as Kendal's modification:

(1) Prepare plain, sugar-free nutrient agar, using 15 grams of agar per liter.

(2) Adjust the reaction to a point just alkaline to litmus.

(3) Flask the agar, 100.0 cc. to a flask, and sterilize in the autoclave.

(4) Prepare a 10.0% solution of basic fuchsin in 96.0% alcohol. This solution is fairly stable if kept away from light.

(5) Prepare a 10.0% aqueous solution of chemically pure anhydrous sodium sulphite (1.0 g. in 10.0 cc. water). This solution does not keep.

(6) Add 1.0 cc. of (4) to 10.0 cc. of (5) and heat in the Arnold sterilizer for 20 minutes. The color of the fuchsin is nearly discharged if the solutions are of proper strength. This solution must be prepared each day—it does not keep.

(7) Add 1.0 g. of C. P. lactose (free from glucose) to 100.0 cc. of agar and place in the autoclave until melted and the lactose is thoroly dissolved.

(8) Add a sufficient volume of (6) (about 1.0 cc.) to impart a faint pink color to the medium.

(9) Pour into sterile Petri dishes and allow to harden in a dark place with the covers partly removed. When cool the medium should be colorless when viewed from above and a very faint pink when viewed

from the edge. The medium must be kept in a dark place because the color is restored by the action of daylight.

(e) Harvey.
  (1) Add 3.7 cc. of a 10% anhydrous sodium carbonate solution to 1000.0 cc. of infusion agar (see variant (v) medium 1661).
  (2) Dissolve 2.0 g. lactose in 25.0 cc. of distilled water.
  (3) Dissolve 0.5 g. anhydrous Na_2SO_3, and 1.0 cc. of a saturated alcoholic basic fuchsin solution in 10.0 cc. distilled water.
  (4) Mix (2) and (3).
  (5) Add (4) to (1).
  (6) Pour in plates.
  (7) Dry the surface of the medium for 15 minutes in the incubator.

(f) Harvey.
  (1) Add 10.0 cc. of a 10.0% crystalline sodium bicarbonate solution to 1000.0 cc. of infusion agar (see variant (v) medium 1661) neutral to litmus.
  (2) Prepare an alcoholic fuchsin solution by adding 1.0 g. basic fuchsin to 20.0 cc. absolute alcohol, allowing it to stand 24 hours, and centrifuge. (A filtered saturated alcoholic fuchsin in the same or half the quantity may be used.)
  (3) Add 10.0 g. c. p. lactose and 5.0 cc. of (2) to (1).
  (4) Add 10.0% freshly prepared crystalline sodium sulphite 25.
  (5) Distribute into test tubes or flasks.
  (6) Sterilize at 115°C.
  (7) Keep in the dark until used.

(g) Harvey.
  (1) Add 10.0 g. lactose to infusion agar (see variant (v) medium 1661) with a reaction of 0.5% alkaline to phenolphthalein.
  (2) Filter (1).
  (3) Add 5.0 cc. of alcoholic fuchsin solution prepared as indicated in step (2) variant (d) above.
  (4) Mix.
  (5) Add 10.0% freshly prepared sodium sulphite 25.
  (6) Sterilize.

References: Endo (1905 p. 109), Reitz (1906 p. 722), Abel (1912 p. 131), Ströszner (1917–18 p. 224), Zipfel (1917–18 p. 477), Levine (1921 p. 114), Harvey (1921–22 pp. 92, 93).

1730. Wurtz's Litmus Lactose Agar

Constituents:

1. Infusion Agar.............. 1000.0 cc.
2. Lactose (2.0%)............ 20.0 g.
3. Litmus

Preparation:
  (1) Add 2.0% lactose to infusion agar with a slightly alkaline (0.5% reaction).
  (2) Tint sterile (1) by the addition of a sufficient quantity of sterile litmus solution.

Sterilization: Sterilize (1) and the litmus solution separately, method not given.

Use: Determine fermentation of lactose.

Variants:

(a) Smith (1905) gave the following method of preparation.
  (1) Add 10.0 g. of c. p. lactose to 1000.0 cc. of sugar free meat infusion agar.
  (2) Add 20.0 cc. of a saturated watery solution of lime-free blue litmus to (1).
  (3) Sterilization not specified.

(b) Heinemann prepared a similar medium as follows:
  (1) Add 1.0% lactose to sugar free infusion agar.
  (2) Tube in 8.0 cc. quantities.
  (3) Add 1.0 cc. of 1.0% sterile litmus solution to each tube before using.

(c) Committee A. P. H. A. (1913).
  (1) Boil 10.0 or 15.0 g. of thread agar in 500.0 cc. of water for half an hour and make up weight to 500.0 g. or digest for 15 minutes.
  (2) Infuse 500.0 g. lean meat for 24 hours with 500.0 cc. of distilled water in a refrigerator.
  (3) Make up lost weight.
  (4) Strain thru cotton flannel.
  (5) Weigh.
  (6) Add 2.0% Witte's peptone and warm on the water bath until solution is complete. Do not heat above 60°C.
  (7) Mix 500.0 cc. (6) and 500.0 cc. of

(1), keeping the temperature below 60°C.
- (8) Titrate and adjust the reaction to neutral to phenolphthalein adding normal HCl or NaOH.
- (9) Heat on a water bath for 40 minutes.
- (10) Make up lost weight.
- (11) Readjust to neutrality if necessary and boil 5 minutes.
- (12) Restore lost weight.
- (13) Filter thru absorbent cotton and cotton flannel.
- (14) Titrate and record final reaction.
- (15) Add 1.0% lactose and sufficient azolitmin solution.
- (16) Tube in 10.0 cc. quantities.
- (17) Sterilize for 15 minutes in the autoclave at 120°C. or for 30 minutes on each 3 successive days.

(d) Giltner gave the following methods of preparation. The medium was used in water analysis.

- I. (1) Prepare infusion agar using equal parts meat infusion and water, with 1.0% peptone and 1.5% agar.
  - (2) Adjust the reaction to +1.0.
  - (3) Add 1.0% lactose and 2.0% azolitmin just before tubing.
  - (4) Tube.
  - (5) Sterilize for 30 minutes on 3 successive days.
- II. (1) Preparation of meat infusion not given.
  - (2) Strain (1) thru a piece of clean cheese cloth.
  - (3) Place 2.0% washed agar in distilled water.
  - (4) Weigh (3).
  - (5) Digest over a free flame.
  - (6) Add distilled water to make up the loss in weight.
  - (7) Add lactose 2.0% and peptone 2.0% to (6) and mix until solution is complete.
  - (8) Add (2) to (7).
  - (9) Adjust the reaction to 0.
  - (10) Add 2.0% azolitmin solution.
  - (11) Boil over a free flame.
  - (12) Distribute in 100.0 cc. lots in 250.0 cc. Florence flasks.
  - (13) Sterilization not specified.

References: Smith (1902 p. 94), (1905, p. 196), Heineman (1905 p. 127), Committee A. P. H. A. (1913 p. 129), Giltner (1921 p. 379, 380).

1731. Bitter's China Blue Malachite Green Agar

Constituents:

1. Infusion agar (containing 2.0 or 3.0% peptone and NaCl)	1000.0 cc.
2. Lactose	20.0 g.
3. China blue (sat. aqueous solution (Höchster))	90 drops
4. Malachite green (0.1% solution, crystalline extra Höchst.)	25.0 cc.

Preparation:
- (1) Prepare meat infusion agar with 2.0 or 3.0% peptone and NaCl.
- (2) Neutralize (1) with N/1 NaOH.
- (3) Add 2.0% lactose.
- (4) Boil a few minutes.
- (5) To each 100.0 cc. of (4) add 9 drops of a saturated (about 10%) watery china blue solution (Höchster Farbwerks 10 g. 2M) by means of a 1.0 cc. pipette.
- (6) Then add to each 100.0 cc. of (5) 2.5 cc. of a 0.1% malachite green (cryst extra Höchst.) solution.
- (7) Pour sterile (6) in thin layers in sterile plates.

Sterilization: Sterilize 10 minutes in the autoclave.

Use: Diagnosis of typhoid fever. Author reported that all acid forming organisms (coli, etc.) gave bright blue colonies. All non-lactose fermenting organisms (typhoid) or alkali forming organisms gave a colorless or yellowish colony.

Reference: Bitter (1911 p. 474).

1732. Guth's Alizarine Lactose Agar (Klimmer)

Constituents:

1. Meat infusion (beef or horse)	1000.0 cc.
2. Agar	30.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Lactose	10.0 g.
6. Alizarin	0.8 g.

Preparation:

(1) Preparation of horse or beef infusion not given.
(2) Dissolve 10.0 g. peptone, 5.0 g. NaCl and 30.0 g. agar in 1.
(3) Clarify.
(4) Neutralize to litmus.
(5) Add 50.0 to 70.0 cc. of tenth normal NaOH and 10.0 g. lactose dissolved in 20.0 or 30.0 cc. water.
(6) Dissolve 0.6 g. NaOH and 0.8 g. alizarine in 100.0 cc. distilled water and boil several minutes.
(7) Add (6) to (5).
(8) Thoroly mix sterile (7) and pour into sterile plates.

Sterilization: Sterilize by steaming for 30 minutes.

Use: Detection of typhoid and paratyphoid bacteria. Author reported that *B. coli* lightened the medium coloring it yellow, typhoid bacilli, paratyphoid and enteritidis bacilli left the medium opaque and formed greyish blue colonies. The addition of malachite green completely inhibited the colon bacteria; however, 40 to 48 hours were required for the development of the typhoid colonies.

Variants: Klimmer added 1.7 cc. of a 0.1% malachite green solution to each 100.0 cc. of medium.

Reference: Klimmer (1923 p. 215).

1733. Harvey's Starch Agar

Constituents:

1. Infusion agar	1000.0 cc.
2. Starch (water soluble)	2.0 g.

Preparation:

(1) See medium 1661, Variant (v) for the preparation of infusion agar.
(2) Add 2.0 g. water soluble starch to 1000.0 cc. of (1).

Sterilization: Not specified.

Use: General culture medium.

Reference: Harvey (1921–22 p. 112).

1734. Gassner's Metachrome Yellow Water Blue Infusion Agar

Constituents:

1. Infusion agar	2000.0 cc.
2. Metachrome Yellow (II RD) (2.0%)	125.0 cc.
3. Water blue (6B extra P) (1.0%)	175.0 cc.
4. Lactose	100.0 g.

Preparation:

(1) Prepare meat infusion agar (yeast may be used instead of meat).
(2) Neutralize to litmus.
(3) Prepare a 2.0% solution of metachrome yellow (II RD) in water.
(4) Boil two minutes.
(5) Prepare a 1.0% water blue (6B extra P) in solution in water and dissolve 100.0 g. lactose in 175.0 cc. of the solution.
(6) Boil (5) 10 minutes.
(7) Add 125.0 cc. of (4) and 175.0 cc. of (6) to 2000.0 cc. (2).

Sterilization: Method not given.

Use: Detection of dysentery or typhoid bacilli. The author reported that *Bact. coli* gave a deep blue medium. Typhoid and dysentery gave a yellowish medium. Medium was green. This medium inhibited neither the typhoid, dysentery nor coli organisms.

Reference: Gassner (1917–18 p. 221).

1735. Torrey's Brom Cresol Purple Lactose Agar

Constituents:

1. Water	1000.0 cc.
2. Beef heart	500.0 g.
3. Peptone	10.0 g.
4. Agar	15.0 g.
5. Lactose	1.0 g.
6. Brom Cresol Purple (Sat. alc. soln.)	0.75 cc.

Preparation:

(1) Boil 500.0 g. finely chopped beef heart (other meat material may be used) in one liter of water for 15 minutes over a free flame
(2) Strain through canton flannel and absorbent cotton.
(3) Make up the loss in filtrate and add 10.0 g. peptone and 15.0 g. flaked agar.
(4) Heat in the Arnold until solution is complete.
(5) Adjust to pH = 7.4 and place in the Arnold for 30 minutes.
(6) Readjust the reaction if necessary.
(7) Filter thru cotton and add 1.0 g.

lactose and a sufficient quantity of a saturated alcoholic solution of brom cresol purple (generally about 0.75 cc.) to give the desired depth of color.

(8) Flask in 200.0 cc. quantities.

Sterilization: Autoclave at 15 pounds pressure for 10 minutes.

Use: Analysis of fecal flora.

Reference: Torrey (1926 p. 353).

1736. Abbott's Glycerol Infusion Agar

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.
3. Peptone (1.0%)	10.0 g.
4. NaCl (0.5%)	5.0 g.
5. Agar (1.0 to 1.5%)	10.0 to 15.0 g.
6. Glycerol (5.0 to 7.0%)	50.0 to 70.0 g.

Preparation:

(1) Add 500.0 g. of chopped lean beef to 1 liter of water and soak for 24 hours, kept at ice box temperature.

(2) Strain thru a coarse towel and press until a liter of fluid is obtained.

(3) Dissolve 10.0 g. (1.0%) peptone, and 5.0 g. (0.5%) NaCl.

(4) Dissolve 1.0 to 1.5% agar in (3) by boiling in a porcelain lined iron vessel. Add 250 to 300.0 cc. water and boil until this volume of water has evaporated, leaving one liter volume.

(5) Place the vessel in a large dish of cold water.

(6) Stir the agar constantly until cooled to 68 to 70°C.

(7) Add the white of one egg which has been beaten up in about 50.0 cc. of water, (a 10.0% dry albumin solution may be used).

(8) Mix (7) thoroly with (6).

(9) Allow to boil slowly for 30 minutes. Do not allow the volume of the liquid to fall below the liter mark.

(10) Filter thru a heavy folded filter paper.

(11) Add 5.0 to 7.0% glycerol to (10).

Sterilization: Sterilize by steam (method not given).

Use: General culture medium.

Variants:

(a) Harvey added 10.0 cc. glycerol, 3.0 cc. normal NaOH to 87.0 cc. of infusion agar (see variant (v) medium 1661). The medium was used to cultivate *V. cholerae.*

(b) Harvey added 50.0 cc. of glycerol to 1000.0 cc. of infusion agar (see variant (v) medium 1661).

Reference: Abbott (1921 p. 132), Harvey (1921–22 p. 87).

1737. Kowalski's Glycerol Lung Infusion Agar

Constituents:

1. Water	2500.0 cc.
2. Lung, calf	1000.0 g.
3. NaCl	18.0 g.
4. Potassium phosphate	9.0 g.
5. $(NH_4)_2SO_4$	9.0 g.
6. Na_2SO_4	25.0 g.
7. Sugar	90.0 g.
8. Peptone	25.0 g.
9. Agar	40.0 g.
10. Glycerol	20.0 to 25.0 g.

Preparation:

(1) Remove the lung of a calf immediately after death. Grind it in a meat grinding machine, keeping as clean as possible during the procedure.

(2) Add two liters of water to 1000.0 g. of finely ground (1). Place in a glass container and boil.

(3) After a half hour, strain thru a sieve. Press out the residue, and refilter the liquid obtained. It is then added to the original filtrate.

(4) Add to the quite clear fluid, 18.0 g. NaCl, 9.0 g. potassium phosphate, 9.0 g. $(NH_4)_2SO_4$, 25.0 g. Na_2SO_4, 90.0 g. sugar, 25.0 g. peptone.

(5) After complete solution of the materials, add 2.0% agar which has been previously soaked for 24 hours in water.

(6) Boil to dissolve the agar, stirring continually.

(7) Neutralize very carefully with equal portions of KOH and NaOH.

(8) Make up to 2500.0 cc. and cool to 58°C.

(9) Add the whites of four hen eggs, beaten to a foam.

(10) Boil a minute, and filter thru a hot water funnel.

(11) To the clear, straw colored filtrate, whose reaction should be neutral, or at the most slightly alkaline, add 8 to 10.0% glycerol with continual shaking.

(12) Distribute in test tubes or flasks.

Sterilization: After 3 appropriate sterilizations (exact details not given), the medium may be slanted or remain upright.

Use: Cultivation of influenza bacilli, tubercle bacilli and other pathogenic organisms.

Reference: Kowalski (1890, p. 245).

1738. Henssen's Glycerol Kidney Agar

Constituents:

1. Water	1000.0 cc.
2. Kidney	500 0 g.
3. Agar	15.0 g.
4. Peptone	10.0 g.
5. Glycerol	30.0 g.
6. NaCl	5.0 g.

Preparation:

(1) Prepare kidney infusion in the same manner as beef infusion (exact method not given).

(2) Dissolve 2, 3, 4, 5 and 6 in (1).

Sterilization: Not specified.

Use: Cultivation of pathogenic organisms.

Reference: Henssen (1895 p. 406).

1739. Harvey's Mannitol Infusion Agar

Constituents:

1. Infusion agar	1000.0 cc.
2. Mannitol	10.0 g.

Preparation:

(1) See variant (v) medium 1661 for preparation of infusion agar.

(2) Add 1.0 g. mannitol to 100.0 cc. of (1).

Sterilization: Not specified.

Use: General culture medium.

Reference: Harvey (1921–22 p. 111).

1740. Omelianski's Formate Agar

Constituents:

1. Meat infusion	1000.0 cc.
2. Agar	20.0 g.
3. Sodium formate	10.0 g.
4. Phenolphthalein solution	200.0 cc.

Preparation:

(1) Prepare a phenolphthalein solution by dissolving 0.5 g. phenolphthalein in 100.0 cc. of a mixture of equal parts of water and alcohol. Dilute with 20 parts water.

(2) Composition of usual infusion broth not given. (A 0.5% meat extract may be substituted for meat infusion.)

(3) Dissolve 2 and 3 in (2).

(4) Add 200.0 cc. of (1) to (3).

Sterilization: Method not given.

Use: To study decomposition of formic acid. Author reported that after 24 hours the agar was colored red near the colonies. After several days the whole plate had turned red.

Reference: Omelianski (1903–04 p. 186).

1741. Harvey's Salicylate Infusion Agar

Constituents:

1. Infusion agar	1000.0 cc.
2. Sodium salicylate (1.0%)	10.0 g.

Preparation:

(1) Add 1.0% sodium salicylate to Harvey's Infusion Agar (see variant (v) of medium 1661).

Sterilization: Not specified.

Use: Cultivation of molds and torulae. Bacterial growth is inhibited.

Reference: Harvey (1921–22 p. 93).

1742. de Gasperi and Savini's Glucose Lactose Agar

Constituents:

1. Water	1000.0 cc.
2. Beef	500.0 g.
3. Agar	12.0 g.
4. Peptone (Witte)	10.0 g.
5. NaCl	5.0 g.
6. Glucose	4.0 g.
7. Lactose	4.0 g.

Preparation:

(1) Mix 500.0 g. finely chopped lean beef with 1000.0 cc. of water, and allow to stand for 24 hours at room temperature.

(2) Boil for 15 minutes over a free flame.

(3) Filter thru a double chardin filter paper and press out the residue.

(4) Add 12.0 g. of finely chopped agar, 10.0 g. of Witte's peptone and 5.0 g. of NaCl, and boil over a free flame, or at 110°C. in the autoclave, until the agar is dissolved.

(5) Cool to about 55°C. and add 4.0 g. of glucose and 4.0 g. lactose and a little

egg albumin stirred up well in a little water.
(6) Add soda solution until the reaction is distinctly alkaline and heat in the autoclave at 120°C. for 20 minutes.
(7) When taken from the autoclave pour immediately upon a Chardin filter paper.
(8) Distribute in about 10–12 cm. layer in a 20 to 22 mm. by 22 cm. test tubes.

Sterilization: Sterilize at 112 to 115°C. for 15 to 20 minutes.

Use: Cultivation of anaerobes. Meier made bacterial counts from milk and whey in a similar medium.

Variants: Meier prepared a similar medium as follows:
(1) Boil 500.0 g. of fat and tendon-free beef in one liter of water.
(2) Filter.
(3) Dissolve 2.5 g. lactose, 2.5 g. glucose, 5.0 g. Witte's peptone, 5.0 g. NaCl and 5.0 g. agar in 1.
(4) Neutralize by the addition of KOH. Add KOH until turmeric paper is turned quite weakly brownish red.
(5) Sterilization not specified.

References: de Gasperi and Savini (1911 p. 248), Meier (1918 p. 454).

1743. Teague and Clurman's Eosin Brilliant Green Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Beef, chopped	500.0 g.
3. Peptone (Witte)	10.0 g.
4. NaCl (C. P.)	5.0 g.
5. Agar	15.0 g.
6. Lactose	10.0 g.
7. Saccharose	10.0 g.
8. 3.0% yellowish eosin solution	20.0 cc.
9. 1.0% brilliant green solution	20.0 cc.

Preparation:
(1) Soak beef in water in the ice chest over night.
(2) Squeeze infusion thru cheese cloth, heat in Arnold sterilizer and pass thru filter paper.
(3) Add 3, 4 and 5 to warm (2). Rub peptone into paste before adding.
(4) Heat in autoclave for 30 minutes at 120°C.
(5) Adjust to +1 with 2N, NaOH.
(6) Heat in Arnold sterilizer for ½ hour.
(7) Adjust again to +1.
(8) Cool to 55°C., clear with egg white and filter thru cotton.
(9) Flask in 100.0 cc. or 200.0 cc. lots.
(10) When ready for use melt sterile (9), add 1.0% lactose and 1.0% saccharose. (1.0 g. to 100.0 cc. medium of each sugar.)
(11) Prepare a 3.0% stock solution of yellowish eosin in distilled water.
(12) Prepare a 1.0% stock solution of brilliant green in 50.0% alcohol, a ⅙% solution in distilled water.
(13) Add 1.0 cc. of (11) and 1.0 cc. of (12) to each 50.0 cc. of medium.
(14) Shake well.
(15) Pour into sterile Petri dishes.

Sterilization: Sterilize (9) by heating in the autoclave at 120°C. for 20 minutes.

Use: Isolation of typhoid bacilli from stools. Author reported that typhoid colonies after 18 hours were grayish in color by reflected light. *B. coli* colonies were red. In transmitted light typhoid were colorless and transparent. Liebig's meat extract cannot be substituted for beef infusion.

References: Teague and Clurman (1916 p. 651), Tanner (1919 p. 54).

1744. Kan-Ichiro Morishima's Lead Acetate China Blue Agar

Constituents:

1. Meat infusion agar (composition not given)	2000.0 cc.
2. Neutral 2.0% solution lead acetate in distilled water	50.0 cc.
3. 1.0% solution China blue	
4. N sodium hydrate solution	
5. Lactose	10.0 g.
6. Glucose	1.0 g.

Preparation:
(1) Preparation or composition of meat infusion agar not given except that it is to be prepared in the usual way.
(2) Clear with egg white.
(3) Titrate while hot to −0.2 to −0.4 with phenolphthalein.
(4) Prepare 2.0% neutral lead acetate in sterile distilled water. Heat for ½ hour at 100°C. in water bath.
(5) Melt (3), and add 5.0 cc. of (4) to

every 100.0 cc. agar. Cool agar to 60°C. or precipitate will be formed.

(6) Transfer to small test tubes, to a depth of 1.5 cc.

(7) Stock solution of 1.0% china blue in distilled water is prepared.

(8) Add 0.4 cc. N sodium hydrate to 10.0 cc. of (7). Heat on water bath for 10 minutes at 100°C. (Color changes from blue to brown.)

(9) 1.2 cc. of (8) is added to 100.0 cc. of (3) reaction −0.2 to −0.4.

(10) Add 1.0% lactose and 0.1% glucose to (9) and heat in water bath at 100°C. for 10 minutes.

(11) Cool to 60°C.

(12) Add to (6) making a second layer in the tubes.

(13) Incubate over night—make stab inoculation.

Sterilization: Not specified.

Use: Differentiation of *B. paratyphosus A*, *B. paratyphotus B*, *B. enteritidis* and *B. coli*.

Variants: The author prepared a similar medium but used 1.0% inositol and 0.1% arabinose instead of 1.0% glucose.

Reference: Kan-Ichiro Morishima (1918 p. 19).

1745. Aronson's Fuchsin Sulphite Agar (Harvey)

Constituents:

1. Infusion agar	1000.0 cc.
2. Na_2CO_3 (10.0% anhydrous soln.)	60.0 cc.
3. Sucrose (20.0% soln.)	50.0 cc.
4. Dextrin (20.0% soln.)	50.0 cc.
5. Fuchsin (sat. alcoholic soln.)	2.5 cc.
6. Na_2SO_3 (10.0% soln.)	25.0 cc.

Preparation:

(1) Prepare a 10.0% anhydrous Na_2CO_3 solution.

(2) Prepare a 20.0% sucrose solution.

(3) Prepare a 20.0% dextrin solution.

(4) Prepare a saturated alcoholic fuchsin solution.

(5) Prepare a 10.0% Na_2SO_3 solution (must be freshly prepared).

(6) Mix 60.0 cc. sterile (1), heated to 45°C. and 1000.0 cc. melted sterile infusion agar (see variant (v) 1661 for preparation), cooled to 45°C.

(7) Steam 15 minutes. The mixture becomes dark brown and very cloudy.

(8) Add 50.0 cc. of sterile (2), 50.0 cc. of sterile (3), 2.5 cc. sterile (4) and 25.0 cc. of sterile (5) to hot (7).

(9) Place the flasks in a sloping position to allow the precipitate formed to settle.

(10) Pour plates carefully, avoiding as far as possible the transference of settled precipitate.

(11) Place the plates to dry in the incubator with their agar surface downwards.

(12) Preserve in the dark for three days before use.

Sterilization: Sterilize (1), (2), (3) and (5) at 100°C.

Use: Isolation of *V. cholerae*.

Reference: Harvey (1921–22 p. 93).

1746. v. Szabóky's Glycerol Lung Agar

Constituents:

1. Water	2000.0 cc.
2. Lung	1000.0 g.
3. Agar	10.0 g.
4. Peptone (Witte)	20.0 g.
5. Glycerol	100.0 g.
6. Glucose	10.0 g.

Preparation:

(1) Boil 1 kilogram of lung with 2 kilograms of water. (Time not specified.)

(2) Filter and dissolve 3, 4, 5 and 6 in the filtrate.

(3) Neutralize to litmus.

(4) Heat again.

(5) Distribute in 20.0 cc. lots into suitable wide sterile test tubes.

Sterilization: Sterilize in the usual manner by short heating in streaming steam (time or number of days not specified).

Use: Cultivation of tubercle bacilli. The author reported that colonies developed in one day when reaction was 0.5% alkaline to 0.5% acid.

Variants: Tubercular lung may be used as well as a normal lung. This tubercular material must be sterilized on 5 days for 2 hours each.

Reference: v. Szabóky (1907 p. 652), Kolle and Wassermann (1912 p. 413).

1747. Hulton-Frankel's Inositol Infusion Agar

Constituents:

1. Infusion agar (3.0%)	1000.0 cc.
2. Dextrin	10.0 g.
3. Inositol	10.0 g.
4. Litmus (Grübler's)	

Preparation:

(1) Method of preparation or exact composition of 3.0% infusion agar not given.

(2) Adjust reaction to about +0.7 acid.

(3) Sterilize in the autoclave (time or pressure not given).

(4) Readjust reaction to +0.7 acid.

(5) Add 2 and 3. (Inositol prepared according to Nelson (Jour. Am. Chem. Soc. 37: 1552, 1915) and dextrin, Merck's C. P.)

(6) Add sterile solution of litmus (Grübler's) till a light violet color is obtained.

(7) Tube and slant after final sterilization.

Sterilization: Partial sterilization given in step (3) above. Sterilize (7) once in the Arnold sterilizer as further heating tends to break up the carbohydrates. Incubate 24 hours and discard tubes showing growth.

Use: To differentiate between typhoid and paratyphoid A and B bacilli. Author reported that typhoid bacillus fermented dextrin-inositol with acid formation. Para-typhoid bacillus B fermented dextrin-inositol with acid and gas formation. Para-typhoid bacillus A did not ferment dextrin-inositol. Shiga-Kruse and Hiss-Russel dysentery types did not ferment dextrin-inositol. Flexner Rosen dysentery types fermented dextrin-inositol. *B. typhi murium* and *B. pullorum* did not ferment dextrin-inositol. *B. aerogenes* fermented dextrin-inositol with gas formation.

Reference: Hulton-Frankel (1918 p. 380).

1748. Robinson and Rettger's Opsine Infusion Agar

Constituents:

1. Beef infusion	1000.0 cc.
2. Agar	15.0 g.
3. Opsine (2.0%)	20.0 g.
4. NaCl	5.0 g.
5. KH_2PO_4	5.0 g.
6. Sodium citrate	2.0 g.
7. $MgSO_4$	2.0 g.
8. Glucose	5.0 g.
9. Glycerol	60.0 g.

Preparation:

(1) Prepare 1000.0 cc. of beef infusion.

(2) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in (1).

(3) Adjust faintly alkaline to litmus.

Sterilization: Sterilize at 12 pounds for 15 minutes.

Use: General culture medium. The author reported that 2.0% opsine gave better general medium than using 1.0%. Medium supported the growth of some pathogenic forms.

Variants: The authors used only 1.0% opsine instead of 2.0%.

Reference: Robinson and Rettger (1918 p. 214).

1749. Lubenau's Lactose Caffeine Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Beef	500.0 g.
3. Agar (2.0 to 3.0%)	20.0 to 30.0 g.
4. Peptone	3.0 g.
5. NaCl (0.5%)	5.0 g.
6. Lactose (0.5%)	5.0 g.
7. Caffeine	
8. Litmus	

Preparation:

(1) Boil 500.0 g. of finely chopped lean beef with one liter distilled water for 30 minutes.

(2) Filter, and make up to one liter.

(3) Add 3, 4, and 5 to (2) and dissolve by boiling in a salt water bath.

(4) Neutralize to litmus.

(5) Boil and filter.

(6) Add 60.0 cc. of sterile litmus solution (preparation not given) to hot sterile (5).

(7) Mix thoroughly, and allow to cool.

(8) Add 110.0 cc. of a sterile 6.0% caffeine solution to (7). Mix well.

(9) Pour in plates.

Sterilization: Sterilize (5), method not given.

Use: Detection of typhoid bacteria.

Variants: Harvey added 5.0 g. lactose, 110.0 cc. of 6.0% caffeine, 60.0 cc. litmus solution containing 6.0% peptone. The lactose, litmus and caffeine were added to the hot sterile nutrient agar.

References: Lubenau (1907 p. 249), Harvey (1921–22 p. 92).

1750. Harvey's Caffeine Endo Agar

Add 33.0 cc. of 10.0% caffeine to 1000.0 cc. of Harvey's modification of Endo agar (see variant (e) medium 1729).

1751. Gäthgen's Caffeine Fuchsin Sulphite Agar (Bezançon)

Constituents:

1. Infusion agar (3.0%)	1000.0 cc.
2. Lactose (10.0% solution)	120.0 cc.
3. Fuchsin	
4. Na_2SO_3 (10.0% soln.)	25.0 cc.
5. Caffeine	3.3 g.

Preparation:

(1) Add 120.0 cc. of a 10.0% soda solution to a liter of neutral 3.0% infusion agar.
(2) Add 120.0 cc. of a 10.0% lactose solution to (1).
(3) Add 10.0 g. of crystalline fuchsin to 100.0 cc. of 96.0% alcohol and allow to stand for 2 hours.
(4) Decant (3) and add 2.5 cc. of the supernatant fluid to sterile (2).
(5) Prepare a 10.0% solution of Na_2SO_3.
(6) Add 25.0 cc. of sterile (5) to (4).
(7) Add 0.33 g. of pure caffeine to each 100.0 cc. quantity.
(8) Pour in plates.

Sterilization: Sterilize (2) at 100°C. for one hour. Sterilize (5) by heating at 80°C. for one hour.

Use: Differentiation of colon typhoid group.

Variants:

(a) Harvey added 33.0 cc. of a 10.0% caffeine solution to 1000.0 cc. of Harvey's modification of Endo agar (see variant (e) 1729).
(b) Klimmer prepared a similar medium as follows:
  (1) Soak 30.0 to 40.0 g. agar in meat water or a 1.0% meat extract solution for several hours.
  (2) Boil for 3 hours.
  (3) Dissolve 10.0 g. peptone and 5.0 g. NaCl in (2).
  (4) Neutralize to litmus.
  (5) Add 7.0 cc. of normal soda solution or 10.0 cc. of a 10.0% soda solution.
  (6) Boil.
  (7) Filter.
  (8) Add 10.0 g. c.p. lactose, 5.0 cc. of a concentrated filtered alcoholic fuchsin solution and 25.0 cc. of a 10.0% freshly prepared sodium sulphite solution.
  (9) Add 0.33% crystalline caffeine to (8).

  Klimmer reported that typhoid, paratyphoid and cholera colonies were blue; colon colonies were red.

References: Bezançon (1920 p. 345), Harvey (1921–22 pp. 92, 93), Klimmer (1923 p. 210).

1752. Viehoever's Basal Glucose Extract Agar

Constituents:

1. Water	500.0 cc.
2. Peptone (Witte)	6.0 g.
3. Meat extract (Liebig's)	4.0 g.
4. NaCl	1.0 g.
5. Glucose	5.0 g.
6. Agar	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Dissolve one of the added nutrients in (1) and adjust the reaction as indicated.
(3) Tube in 5.0 cc. lots.

Sterilization: Method not given.

Use: To study spore production by urea splitting organisms, *Bac. probatus*.

Added nutrients: The author prepared one of the mediums as indicated:

(a) Neutral basal medium + 0.25% Na_2CO_3.
(b) Neutral basal medium + 1.0% $CaCO_3$.
(c) Neutral basal medium + 2.0% urea + 0.3% $(NH_4)_2CO_3$.

Variants: The author gave the following variants:

(a) Basal medium with 0.3, 0.2 or 0.1 concentration of nutrients + 0.1% Na_2CO_3.
(b) Basal medium with 0.3 concentration of nutrients + 0.05, 0.25 or 1.0% Na_2CO_3.
(c) Basal medium with 0.3 concentration

of nutrients + 2.0% or 0.2% urea + 0.1% Na_2CO_3.

(d) Basal medium with 0.3 concentration of nutrients + 0.2% $(NH_4)_2CO_3$ + 0.1% Na_2CO_3.

Reference: Viehoever (1913 p. 214).

1753. Bacto Dextrose Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Beef extract (Bacto)	3.0 g.
3. Peptone (Bacto)	5.0 g.
4. Glucose (Bacto)	10.0 g.
5. Agar (Bacto)	15.0 g.

Preparation:

(1) Dissolve 33.0 g. of Bacto Dextrose Agar (Dehydrated) in 1000.0 cc. distilled water by boiling or preferably autoclaving for 10 minutes at 15 pounds.

(2) If sterilized 10 minutes at 15 pounds pH = 7.4±.

Sterilization: Sterilize in the usual manner.

Use: General culture medium. Add 8.0 g. NaCl per liter to prevent hemolysis when using as a base for blood agar.

Reference: Digestive Ferments Co., (1925 p. 11).

1754. Henneberg's Glucose Extract Agar

Constituents:

1. Water	1000.0 cc.
2. Glucose	20.0 to 50.0 g.
3. Meat extract	10.0 g.
4. Peptone	10.0 g.
5. NaCl	2.0 g.
6. Agar	10.0 g.

Preparation:

(1) Dissolve 2,3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of acetic acid bacteria, *B. pasteurianum*, *B. oxydans*, *B. aceti*, *B. kützingianum*. Other investigators cultivated a large variety of organisms on similar media.

Variants:

(a) Gottheil cultivated organisms found in the soil and on the roots and rhizomes of plants:

(1) Dissolve 2.0 g. NaCl, 8.0 g. meat extract, Liebig's, and 12.0 g. Witte's peptone in 500.0 cc. of water.

(2) Neutralize with concentrated Na_2CO_3 solution.

(3) Heat for a time in a sterilizer.

(4) Filter.

(5) Soak 16.0 g. of agar in 500.0 cc. water for about three hours.

(6) Mix (4) and (5).

(7) Allow to stand for 3 hours at 100°C.

(8) Neutralize once more with Na_2CO_3 and heat for a short time.

(9) Filter.

(10) Add 10.0 g. glucose and sterilize on three successive days.

(b) Bredeman cultivated *Bac. amylobacter* and prepared the medium as follows:

(1) Wash 215.0 g. of agar in running tap water for three or four hours.

(2) Add water to (1) until the total weight is 10 kilograms. Dissolve the agar in the water.

(3) Clarify with egg white and filter.

(4) Dissolve 7.2 g. Witte's peptone, 6.0 g. dextrose, 4.8 g. Liebig's meat extract and 1.2 g. NaCl in 200.0 cc. of water.

(5) Adjust the reaction so that it is slightly alkaline.

(6) Boil and filter.

(7) Liquify 400.0 g. of (3).

(8) Mix (7) and (6).

(9) Sterilize on three successive days in streaming steam. (The author also prepared the medium by dissolving the constituents in the water and adjusting the reaction to a slight alkalinity to litmus.)

(c) Zikes used medium containing 1.0% agar, 1.0% peptone, 1.0% meat extract, 0.5% NaCl and 0.25% glucose and cultivated apiculatus yeast, *Torula alba, Torula Molischiana. Mycoderma cervisiae. Blastoderma salmonicolor.*

(d) Bachmann cultivated obligate anaerobes using Gottheil's medium, but used 1.0% peptone instead of 1.2%.

e) Burckhardt and Enriquez cultivated diphtheria bacilli on a medium prepared as follows:

(1) Dissolve 10.0 g. agar, 10.0 g. peptone, 10.0 g. meat extract, 5.0 g. NaCl in 1000.0 cc. of water.

(2) Neutralize to litmus.

(3) Add 15.0 g. glucose.
(4) Add 12.5 cc. of N/1 soda solution.
(5) Boil for 30 minutes.
(6) Allow to slowly solidify.
(7) Remove from the container in a solid state and cut away the bottom part containing the sediment.
(8) Melt the clear top portion in a steamer and pour onto a filter.
(9) Distribute into small test tubes (10 mm. in diameter and 12 cm. high).
(10) Sterilize two times (method not specified).

(f) Robinson and Rettger used a medium containing 2.0% opsine, 0.5% NaCl, 1.5% agar, 10.0 g. glucose, and 5.0% Liebig's meat extract.

(g) Dawson studied the variation of *B. coli* on a medium composed of 2.5 g. peptone, 10.0 g. meat extract, 10.0 g. glucose and 20.0 g. agar (10.0 g. glycerol may be added).

(h) Stitt prepared the medium as follows:
(1) Mix 3.0 g. meat extract, 10.0 g. peptone, 5.0 g. NaCl, 10.0 g. glucose and 15.0 g. agar with 1000.0 cc. water containing the whites of one or two eggs.
(2) Boil in a rice cooker until solution is complete.
(3) Filter.
(4) Sterilization not specified.

References: Henneberg (1898 p. 18), Gottheil (1901 p. 432), Bredeman (1909 p. 409), Zikes (1911 p. 148), Bachmann (1912–13 p. 7), Burckhardt and Enriquez (1917–18 p. 16), Robinson and Rettger (1918 p. 202), Dawson (1919 p. 142), Stitt (1924 p. 38).

1755. Oldekop's Neutral Red Glucose Extract Agar

Constituents:

1. Distilled water	500.0 cc.
2. Meat extract (Liebig's)	5.0 g.
3. NaCl	2.5 g.
4. Peptone (Witte siccum)	10.0 g.
5. Agar	1.5 g.
6. Neutral red, concentrated solution	1.0 or 2.0 cc.
7. Glucose (0.15%)	0.75 g.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Add soda solution until a weak alkaline reaction is obtained.
(3) Boil one hour and filter.
(4) Adjust the reaction once more and add 0.3% agar to (3).
(5) Dissolve the agar by boiling in the autoclave for one hour.
(6) Filter while hot.
(7) Add 1.0 or 2.0 cc. of a concentrated neutral red solution (exact concentration not given).
(8) Dissolve 0.15% glucose in (7).
(9) Distribute into 5.0 cc. lots in test tubes.

Sterilization: Sterilize for 15 minutes to two hours in the autoclave (pressure not specified.)

Use: Differentiation between coli and typhoid organisms. Author reported that coli types produced decolorization and fluorescens after 24 hours. Typhoid colonies caused no change in color of the medium.

References: Oldekop (1904 p. 123), Klimmer (1923 p. 210).

1756. Bacto Purple Lactose Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Beef extract (Bacto)	3.0 g.
3. Peptone (Bacto)	5.0 g.
4. Agar (Bacto)	10.0 g.
5. Lactose (Bacto)	10.0 g.
6. Dibromcresolsulphonephthalein	0.025 g.

Preparation:
(1) Dissolve 29.0 g. of Bacto Purple Lactose Agar (Dehydrated) in 1000.0 cc. of distilled water by boiling or autoclaving.
(2) Restore the loss if necessary.
(3) If sterilized at 15 pounds pressure for 20 minutes, pH = 6.5±.

Sterilization: Sterilize in the usual manner.

Use: To determine lactose fermentation. Acid colonies are yellow, alkali producers are purple. Author reported that this medium is superior to 1757.

Reference: Digestive Ferments Co. (1925 p. 12).

1757. Bacto Litmus Lactose Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Beef extract (Bacto)	3.0 g.
3. Peptone (Bacto)	5.0 g.
4. Agar (Bacto)	10.0 g.
5. Lactose (Bacto)	10.0 g.
6. Azolitmin	1.0 g.

Preparation:

(1) Dissolve 29.0 g. of Bacto Litmus Lactose Agar (Dehydrated) in 1000.0 cc. of distilled water by boiling or autoclaving.

(2) Restore the loss if necessary.

(3) If sterilized at 15 pounds for 20 minutes pH = 7.0±.

Sterilization: Sterilize in the usual manner.

Use: To determine fermentation of lactose.

Reference: Digestive Ferments Co. (1925 p. 12).

1758. A.P.H.A. Litmus Lactose Extract Agar (1917)

Constituents:

1. Distilled water	1000.0 cc.
2. Beef extract	3.0 g.
3. Peptone	5.0 g.
4. Agar	12.0 g.
5. Lactose (1.0%)	10.0 g.
6. Litmus	100.0 cc.

Preparation:

(1) Add 3.0 g. beef extract, 5.0 g. peptone and 12.0 g. agar, dried for 30 minutes at 105°C. before weighing, to 1000.0 cc. distilled water.

(2) Boil over a water bath until solution is complete. Make up lost weight.

(3) Cool to 45°C. in a cold water bath and warm to 65°C. in the same bath without stirring.

(4) Restore lost weight, titrate and adjust the reaction to neutral to phenolphthalein.

(5) Filter thru cloth and cotton until clear.

(6) Add 1.0% lactose.

(7) Tube in 10.0 cc. quantities.

(8) Add 1.0 cc. of sterilized litmus or azolitmin solution to each 10.0 cc. of sterile agar just before it is poured into the Petri dish. The mixture may occur in the plate itself if desired.

Sterilization: Sterilize (6) in the autoclave at 15 pounds pressure (120°C.) for 15 minutes.

Use: To determine the fermentation of lactose.

Variants: The following investigators prepared similar media as indicated below:

(a) Meyer.

(1) Dissolve 10.0 g. peptone, 10.0 g. meat extract and 30.0 g. agar (3.0%) in 1000.0 cc. of water.

(2) Distribute in 400.0 cc. portions.

(3) Sterilization not given.

(4) To each 400.0 cc. lot add 16.0 g. of a sterile 25.0% lactose solution and 12.0 cc. of a sterile 8.0% litmus solution.

(5) Pour into sterile plates and allow to harden in open air.

(b) Bendick (Tanner).

(1) Dissolve 15.0 g. agar, 5.0 g. beef extract, 10.0 g. NaCl and 10.0 g. peptone in 1000.0 cc. of water.

(2) Clarify with egg and filter.

(3) Adjustment of reaction is not necessary.

(4) Distribute in 250.0 cc. lots in 500.0 cc. flasks.

(5) Add 1.0 g. $CaCO_3$ to each flask.

(6) Sterilize—method not given.

(7) Add 25.0 cc. of Kahlbaum's aqueous litmus solution and 5.0 g. lactose to each flask.

(8) Tube by pouring directly into the tube from the flask, keeping the contents mixed well.

(c) Stitt.

(1) Prepare nutrient agar by dissolving 5.0 g. Liebig's extract, 5.0 g. NaCl, 10.0 g. peptone and 30.0 g. agar in 1000.0 cc. water.

(2) Adjust the reaction to 0 or +0.2 to phenolphthalein.

(3) Keep this agar base in 100.0 cc. quantities in Erlenmeyer flasks.

(4) Color 100.0 cc. of neutral agar base to a lilac color with azolitmin.

(5) Add 5.0 cc. of a hot freshly prepared 20.0% lactose solution in distilled water.

(6) Tube in 10.0 cc. quantities or distribute in 50.0 or 100.0 cc. Erlenmeyer flasks.

(7) Sterilize in the autoclave at 10

pounds for 15 minutes, or in the Arnold.

- (d) Conradi-Drigalski (Stitt).
  - (1) Add 1.0 cc. of a 1 to 100 solution of crystal violet in distilled water to 100.0 cc. of the medium as prepared by Stitt above (variant (c)).

References: Committee A.P.H.A. (1917 p. 97), Meyer (1917 p. 238), Tanner (1919 p. 48), Committee A.P.H.A. (1920 p. 97), Stitt (1923 p. 49).

1759. Endo's Fuchsin Sulphite Agar (Heinemann)

Constituents:

1. Extract agar (3.0%)	1000.0 cc.
2. Lactose	10.0 g.
3. Fuchsin (alc. soln.)	5.0 cc.
4. Na_2SO_3 (10.0% soln.)	25.0 cc.
5. NaOH (10.0% soln.)	10.0 cc.

Preparation:

- (1) Prepare a medium from 2, 3 and 4 using 3.0% extract agar as a base.
- (2) Add 10.0 cc. of a 10.0% NaOH solution.

Sterilization: Not specified.

Use: Isolation of typhoid bacilli. Coli colonies large and red, typhoid colonies small, colorless and bluish.

Variants: The following authors have prepared similar media as indicated:

- (a) Klinger.
  - (1) Add 20.0 g. Liebig's meat extract, 20.0 g. Witte's peptone, 10.0 g. NaCl and 80.0 g. agar to 1000.0 cc. of water.
  - (2) Heat for either 3 hours in streaming steam, or 2 hours at 110°, or one hour at 120°C.
  - (3) Filter thru a thick layer of cotton.
  - (4) Neutralize to litmus.
  - (5) Add 20.0 cc. of a sterile 10.0% soda solution to (4).
  - (6) Add 20.0 g. of chemical pure lactose to (5).
  - (7) Add 10.0 g. of crystalline fuchsin to 100.0 cc. of 96% alcohol and allow to stand for 20 hours.
  - (8) Pour off the saturated solution.
  - (9) Add 10.0 cc. of (8) to (6).
  - (10) Add 50.0 cc. of a freshly prepared 10.0% Na_2SO_3 solution to (9).
  - (11) Mix well.
  - (12) Distribute in 200–400 cc. lots and store in the dark.
  - (13) Sterilization not specified.
- (b) Kastle and Elvove.
  - (1) Dissolve 10.0 g. Liebig's extract, 10.0 g. peptone and 5.0 g. NaCl in 1000.0 cc. distilled water by heat.
  - (2) Cool and add 40.0 g. powdered agar. Allow agar to settle.
  - (3) Place in Arnold and cook 3 hours.
  - (4) Neutralize to litmus with Na_2CO_3.
  - (5) Filter thru cotton on a Buchner filter or allow to settle, rejecting turbid bottom part.
  - (6) Add 10.0 cc. of a sterile 10.0% Na_2CO_3 solution to (5) after filtering.
  - (7) This medium may be stored in 100–200 or 400.0 cc. flasks until desired for use.
  - (8) When desired for use melt (7) and to each liter add c.p. 10.0 g. lactose and 5.0 cc. of a 10.0% alcoholic fuchsin solution (freshly prepared).
  - (9) Prepare a 10.0% alcoholic fuchsin solution by shaking 10.0 g fuchsin (not acid fuchsin) with 100.0 cc. of 96% alcohol, allow to stand 24 hours. Decant supernatant fluid and filter this fluid each time immediately before use.
  - (10) After fuchsin and sulphite have been added to (7), shake vigorously and place unstoppered in sterilizer for 5 to 10 minutes to allow foam to settle.
  - (11) Add 25.0 cc. of a freshly prepared sterile 5.0% anhydrous Na_2SO_3 solution to (10) and mix by rotating flask.
  - (12) Sterilize in Arnold for a few minutes and pour in Petri dishes while steaming hot. Solidified medium nearly colorless to transmitted light—and rose or flesh to reflected light.
- (c) Kendall and Day.
  - (1) "Dust" 15.0 g. powdered agar upon the surface of cold tap water. Allow to settle into the medium before heat is applied or other ingredients added.

(2) Add 10.0 g. Witte's peptone and 3.0 g. Liebig's meat extract to (1) and cook in a double boiler for one hour.
(3) Make faintly alkaline to litmus by cautious addition of NaOH.
(4) Cook 15 minutes to set the reaction.
(5) Filter thru absorbent cotton.
(6) Distribute in 100.0 cc. lots in flasks and sterilize in the autoclave.
(7) Prepare a 10.0% solution of fuchsin in 96.0% alcohol.
(8) Prepare a 10.0% solution of sodium sulphite in water.
(9) Add 1.0 cc. of (7) to 10.0 cc. of (8) and heat in the Arnold sterilizer for 20 minutes.
(10) Add 1.0% lactose (which must be chemically pure) to (6).
(11) Heat in Arnold sterilizer until the agar is melted and the lactose thoroly distributed in it.
(12) Add (9) (decolorized fuchsin solution) in the proportion of 1.0 cc. to each 100.0 cc. of the medium.
(13) Mix thoroly and pour into sterile plates.
(14) Allow to harden (with covers removed) in the incubator for 30 minutes. The plates are now ready for inoculation.

(d) Kinyoun and Deiter.
(1) Mix 80.0 g. Witte's peptone, 40.0 g. NaCl and 160.0 g. powdered agar with sufficient water to make a smooth paste.
(2) Add sufficient water to make 8000.0 cc.
(3) Add 80.0 g. Liebig's meat extract (not necessary to mix).
(4) Place in the Arnold sterilizer and steam until solution is complete. Usually requires about an hour.
(5) Cool to 55°C. and titrate to +1 using phenolphthalein as an indicator.
(6) Place in tall beakers, steam an hour and allow to solidify. (Allow the beakers to stay in the steamer over night.)
(7) Remove the solid agar from the beaker and cut away all the agar containing sediment.
(8) Cut the clear agar in small pieces, replace in the beakers and melt again.
(9) Cool to 55°C.
(10) Place 18 lots of 10.0 cc. each in an equal number of sterile test tubes and divide the tubes in two series.
(11) To one series add 0.01, 0.03, 0.05, 0.07, 0.09 and 0.1 cc. of normal HCl.
(12) To the other series add 0.0, 0.01, 0.03, 0.05, 0.07, 0.09, 0.1, 0.3, 0.5, 0.7 and 0.9 cc. of 2.5% solution of Na_2CO_3.
(13) To each tube of (11) and (12) add 1.0 cc. of a 10.0% solution of crystalline lactose, 1.0 cc. of a 2.5% solution of Na_2SO_3, freshly prepared, and 1.0 cc. of a half saturated alcoholic solution of basic fuchsin.
(14) Pour each tube into sterile petri dish and allow to harden.
(15) Inoculate one-half the surface of each plate with a 24 hour broth culture of typhoid bacilli, and the other half of each plate with a similar coli culture.
(16) Incubate for 24 hours.
(17) The plate showing most typical luxuriant growth of both the coli and typhoid is chosen as the proper amount of acid or alkali to add to the remainder of the medium.
(18) Add the necessary amount of acid or alkali to the entire lot of agar (9).
(19) Distribute in 100.0 cc. lots in flasks.
(20) Sterilize in the Arnold for an hour on two successive days.
(21) Store until ready for use.
(22) When ready for use add 1.0 g. crystalline lactose to each flask of melted agar.
(23) When the lactose has dissolved add 5.0 cc. of a 5.0% solution of anhydrous Na_2SO_3, freshly prepared and still hot to each flask.
(24) Add 1.0 cc. of a half saturated alcoholic solution of basic fuchsin to each flask.
(25) Mix well.
(26) Pour into plates.

(e) Committee A.P.H.A. (1913).
  (1) Add 30.0 g. of agar to one liter of cold water by sifting successive small portions upon the surface and allowing them to settle.
  (2) Add 10.0 g. Witte's peptone, 5.0 g. meat extract to (1) and boil until solution is complete.
  (3) Neutralize to litmus by adding Na_2CO_3.
  (4) Distribute in 100.0 cc. quantities.
  (5) Prepare a 10.0% solution of Na_2SO_3 in water.
  (6) Prepare a 10.0% basic fuchsin solution in 96% alcohol.
  (7) Add 2.0 cc. of (6) to 10.0 cc. of (5) and steam for a few minutes in the Arnold steamer.
  (8) Add 1.0 g. chemically pure lactose to each 100.0 cc. of sterile (4).
  (9) Melt (8) and add 0.5 cc. of (7).
  (10) Pour in plates.
  (11) Sterilize (4) for 2 hours in streaming steam.

(f) Robinson and Rettger.
  (1) Dissolve 25.0 g. powdered agar, 10.0 g. Peptone (American brand), and 5.0 g. Liebig's meat extract in 1000.0 cc. water.
  (2) Make neutral to litmus.
  (3) Steam in the autoclave at 12 to 15 pounds extra pressure for 35 to 40 minutes.
  (4) Filter thru absorbent cotton and cheese cloth.
  (5) Add Na_2CO_3 and heat for 10 minutes on boiling water bath. Reaction 0.1 to phenolphthalein.
  (6) Add 10.0 g. lactose and 5.0 cc. of a saturated alcoholic solution of fuchsin to hot liquid. The medium is now brilliant red.
  (7) Add 10.0 cc. of a 10.0% anhydrous sodium bisulphite solution.
  (8) Distribute into large test tubes in 20.0 cc. lots.
  (9) Sterilize for 5 to 7 minutes at 10 pounds extra pressure.

(g) Roddy.
  (1) Prepare a 3.0% extract agar.
  (2) Liquify (1) in the Arnold steam sterilizer.
  (3) Dissolve 10.0 g. lactose in (2).
  (4) Adjust the reaction. Best results are obtained when the medium is just slightly alkaline to litmus.
  (5) Add exactly 3.0 cc. of a saturated alcoholic fuchsin solution and 30.0 cc. of a 10.0% solution of anhydrous Na_2SO_3 (or 3.0 cc. of a saturated aqueous solution of Na_2SO_3 may be added).
  (6) Tube.
  (7) Sterilize for 20 minutes on each of 3 successive days in an Arnold sterilizer.

(h) Committee A.P.H.A. (1917–1920).
  (1) Add 5.0 g. beef extract, 10.0 g. peptone and 30.0 g. agar, dried at 105°C. for one hour before weighing to 1000.0 cc. of distilled water.
  (2) Boil on a water bath until all the agar is dissolved and then make up the water lost by evaporation.
  (3) Cool to 45°C. in a cold bath, then warm to 65°C. in the same bath without stirring.
  (4) Make up lost weight.
  (5) Titrate and if the reaction is not already between neutral and +1, adjust to neutral.
  (6) Filter thru cloth and cotton until clear.
  (7) Distribute in 100.0 cc. (or larger) quantities in flasks.
  (8) Sterilize in the autoclave at 15 pounds pressure (120°C.) for 15 minutes after the pressure reaches 15 pounds.
  (9) Prepare a 10.0% solution of basic fuchsin in 95.0% alcohol, allow to stand 20 hours, decant and filter the supernatant fluid. This is a stock solution.
  (10) When ready for use prepare a 10.0% anhydrous sodium sulphite.
  (11) To each 10.0 cc. of (8) add 2.0 cc. of (7) and steam for 5 minutes in the Arnold or water bath.
  (12) To each 100.0 cc. of agar, add 1.0 g. of lactose, and dissolve in streaming steam or on a water bath and 0.5 cc. of (9).
  (13) Pour into Petri dishes and allow to harden thoroly in the incubator before use.

(i) Krumwiede, Kohn, Kuttner and Schumm.

(1) Dissolve 25.0 g. agar, 10.0 g. peptone and 5.0 g. meat extract in 1000.0 cc. water by heating over a gas stove.
(2) Adjust reaction neutral to litmus using 10.0% Na_2CO_3 solution.
(3) Add 10.0 cc. of 10.0% Na_2CO_3.
(4) Cool to 45°C. and add one egg.
(5) Autoclave for 30 minutes at 15 pounds pressure.
(6) Filter and add 10.0 g. lactose (boil to dissolve).
(7) Add 10.0 cc. of 10.0% Na_2CO_3 solution, giving a reaction of −0.3 to phenolphthalein (hot titration).
(8) Distribute in bottles in 100.0 cc. lots.
(9) Autoclave for 10 minutes at 10 pounds pressure.
(10) To melted (9) just before use add 0.5 cc. of a saturated alcoholic solution of fuchsin and 1.0 cc. of a 10.0% solution of amorphous sodium bisulphite (per 100.0 cc.?).

(j) Tanner (Hygienic Laboratory).

(1) Add 3.7 cc. of a 10.0% anhydrous Na_2CO_3 solution to 1000.0 cc. of a 3.0% extract agar with a reaction of +0.5 to phenolphthalein.
(2) Flask in 200.0 cc. quantities.
(3) Dissolve 2.0 g. of chemically pure lactose in 25.0 to 30.0 cc. of distilled water with the aid of gentle heat.
(4) Dissolve 0.5 g. anhydrous sodium sulphite in 10 to 15.0 cc. distilled water.
(5) Add 1.0 cc. of a saturated solution of basic fuchsin in 95.0% alcohol to (4).
(6) Add (5) to (3).
(7) Add (6) to 200.0 cc. of melted agar.
(8) Pour into plates.
(9) When hard, dry for 15 minutes in the incubator.

(k) Kendall (Tanner).

(1) Prepare plain sugar free extract agar using 15.0 g. agar per liter.
(2) Adjust to slightly alkaline to litmus.
(3) Distribute in 100.0 cc. quantities in flasks.
(4) Prepare a 10.0% basic fuchsin solution in 96% alcohol.
(5) Prepare a 10.0% solution of chemically pure anhydrous sodium sulphite (1.0 g. in 10.0 cc. water).
(6) Add 1.0 cc. of (4) to 10.0 cc. of (5), freshly prepared.
(7) Heat in the Arnold sterilizer for 20 minutes.
(8) Add 1.0 g. lactose to 100.0 cc. of (3) and place in the autoclave until melted and the lactose is dissolved.
(9) Add about 1.0 cc. of (7) to each 100.0 cc. flask.
(10) This should give a pink color.
(11) Pour into sterile Petri dishes.

(l) Bezançon.

(1) Add 120.0 cc. of a 10.0% soda solution to a liter of neutral 3.0% extract agar.
(2) Add 120.0 cc. of a 10.0% lactose solution to (1).
(3) Sterilize at 100° for one hour.
(4) Add 10.0 g. of crystalline fuchsin to 100.0 cc. of 96% alcohol and allow to stand for 2 hours.
(5) Decant (4) and add 2.5 cc. of the supernatant fluid to (3).
(6) Sterilize a 10.0% solution of Na_2SO_3 at 80° for one hour.
(7) Add 25.0 cc. of (6) to (5).
(8) Pour in plates.

(m) Giltner.

(1) Prepare an ordinary agar from 5.0 g. Liebig's meat extract, 5.0 g. NaCl, 10.0 g. peptone, 30.0 g. agar and 1000.0 cc. water.
(2) Adjust the reaction to 0 or +0.2%.
(3) Store in 100.0 cc. quantities in Erlenmeyer flasks.
(4) Melt a flask of (3), and add 6 drops of a saturated alcoholic solution of basic fuchsin (4.0 g. fuchsin to 100.0 cc. of 95% alcohol).
(5) Mix well.
(6) Add about 18 to 20 drops of a freshly prepared 10.0% solution of Na_2SO_3.
(7) Add 5.0 cc. of a freshly hot 20.0% aqueous solution of lactose to the hot agar.
(8) Pour into plates.

(n) Savage.

(1) Peptone 10.0 g., Liebig's extract of beef, 10.0 g., sodium chlorid 5.0 g., are boiled up in an enamalled dish with one liter of distilled water. The mixture is then poured into a flask, 30.0 g. of powdered agar added, and the whole heated in the autoclave at 115°C. for one hour. The flask is removed, and after cooling to about 60°C., the white of one egg mixed with a little distilled water is added. The contents are coagulated by heating in current steam in the usual way, filtered, and the filtrate made up to 1 liter. The mixture is made neutral, litmus paper being used as the indicator. Then 19.0 cc. of normal sodium carbonate solution and 10.0 g. c.p. lactose are added. The flask is replaced for 30 minutes in the steam sterilizer. Almost invariably there is a considerable precipitate and the mixture has to be again filtered.

(2) Seven cc. of the fuchsin solution (see below) are added, followed by 25.0 cc. of a quite freshly prepared 10.0% sodium sulphite solution. The mixture becomes much less red, but is not immediately decolorized. It is then distributed conveniently into small flasks, each containing 50.0 to 60.0 cc. of media, and sterilized in current steam for 2 days, 30 minutes each day.

(3) The fuchsin solution is made as follows: 3.0 g. powdered crystalline fuchsin are placed in a dry flask, and 60.0 cc. of absolute alcohol are added. The contents are thoroly mixed, and the flask tightly stoppered, allowed to stand for exactly 24 hours at 20° to 22°C. The alcoholic extract is then decanted and preserved in a clean glass-stoppered bottle. Made in this way a uniform fuchsin extract is obtained which keeps well, and the same quantity of fuchsin is added each time a fresh batch of medium is prepared; a matter of much importance.

(4) The medium must be stored in the dark, since light gradually turns it red. When solidified it is almost free from color.

(o) U. S. A. Med. Dept.

(1) Into a container put 1 liter of tap water, marking the level of the fluid. Add 30.0 g. thread agar, 10.0 g. peptone, 5.0 g. NaCl, 5.0 g. beef extract. Cook until dissolved—it is best to autoclave 30 minutes at 15 pounds; filter thru sterile gauze or cotton. If necessary clear with egg. For this purpose, for each liter beat up the white of one egg with 10.0 cc. of warm water until the egg is well mixed. Add this to agar cooled to 55°C., mix thoroly, heat for 30 minutes or autoclave and filter thru cotton.

(2) This stock agar is kept on hand in quarter-liter flasks or bottles. Agar is standardized just before use and reaction adjusted to 0.2% acid to phenolphthalein. Before use, fuchsin and sodium sulphite are added. A filtered, saturated solution of basic fuchsin in 95% alcohol is kept on hand. A 10.0% solution of dry sodium sulphite crystals in sterile water is freshly made.

(3) Teague has shown that a 10.0% solution of crystalline sodium sulphite can be heated for 20 minutes at 15 pounds pressure with practically no change, and that the 10.0% sodium sulphite solution covered with a layer of liquid petroleum about one cm. thick and sterilized in the autoclave can be kept at room temperature for 3 weeks and probably much longer with but very slight change.

(4) One and eight-tenths cc. of fuchsin solution is added per liter to the agar. After this has been done the sodium sulphite solution is added gradually until the hot agar is almost decolorized—usually about 25.0 cc. to the liter. A pale rose color should be present in the hot agar which fades to a very faint pink on cooling; 10.0 g. of

lactose is dissolved in a little water, filtered and added to each liter.

Various fuchsin solutions may differ and the absolute quantities given above may not be exactly the proper balance in separate lots. These are aproximate, however, and the proper balance can easily be attained by a little preliminary testing in which sodium sulphite solution is added to small quantities of fuchsin solution in a test tube.

The finished product is poured into large sterile Petri dishes. The cover is left off until the agar is hard. Smears are made on these plates.

It is helpful to lay a piece of filter paper into the lid of the petri plate in order to absorb liquid evaporating from the agar in the incubator. If there is not enough filter paper for this, the plate should be placed upside down in the incubator.

(p) Committee A.P.H.A. (1923)—Same as for 1917–1920 (variant (h) above) but adjust the reaction to an alkalinity slightly higher than required instead of to +1.0.

(q) Stitt.

(1) Prepare nutrient agar using 5.0 g. Liebig's meat extract, 5.0 g. NaCl, 10.0 g. peptone and 30.0 g. agar per 1000.0 cc. water. (Method not given.)

(2) Adjust the reaction to 0 or +0.2 to phenolphthalein.

(3) Keep this agar base in 100.0 cc. quantities in Erlenmeyer flasks.

(4) When ready for use melt the agar, and while liquid add 6 drops saturated alcoholic solution of basic fuchsin to each flask.

(5) Then add about 20 drops of a freshly prepared 10.0% solution of sodium sulphite. This decolorizes the intense red color to a light rose pink.

(6) Add 5.0 cc. of a freshly prepared hot aqueous 20.0% solution of chemically pure lactose to each flask.

(7) Mix thoroly.

(8) Pour into plates.

(r) Kligler and Defandorfer (Park, Williams and Krumwiede).

(1) Prepare a beef extract agar. The reaction should be from pH = 7.6 to 7.8.

(2) Sterilize (1) in 100.0 cc. quantities (method not given).

(3) When desired for use add 1.0 g. of lactose to each bottle.

(4) Melt the agar. This sterilizes the lactose.

(5) Add 1.0 cc. of a saturated alcoholic solution of fuchsin to 10.0 cc. of a freshly prepared solution of sodium bisulphite.

(6) Heat in the Arnold sterilizer for 30 minutes.

(7) Add 0.5 cc. of (6) to each bottle of melted agar.

(8) Pour in plates and allow to harden without the covers.

(9) Dry in the incubator for 30 minutes, protecting the plates from dust.

(s) Committee A.P.H.A. (1925).

(1) Add 5.0 g. beef extract, 10.0 g. peptone and 30.0 g. agar (undried market product) to 1000.0 cc. distilled water.

(2) Boil until the agar is dissolved. This may be done over a free flame with constant stirring or place in the autoclave, in a straight walled container, and autoclave at 15 pounds pressure for 15 minutes. Shut off the steam and allow the agar to stand in the autoclave until it has solidified. Then dump the solidified agar on a clean paper and cut away the sediment. Cut the agar into small pieces and melt. If the agar is dissolved over a free flame, it is necessary to filter thru cotton or cloth.

(3) Adjust the reaction so that the pH is between 7.8 and 8.2.

(4) Distribute in 100.0 cc. lots in flasks.

(5) Sterilize in the autoclave at 15 pounds pressure for 15 minutes.

(6) Prepare a 10.0% solution of basic

fuchsin in 95% alcohol and allow to stand 24 hours.

(7) Decant and filter the supernatant liquid.

(8) Add to each 100.0 cc. of sterile melted (5), 1.0% of chemically pure lactose in sterile solution 0.5 cc. of (7), and 0.125 g. anhydrous Na_2SO_3, dissolved in a small amount of hot distilled water. Mix thoroly.

(9) Pour in plates.

References: Heinemann (1905 p. 130), Klinger (1906 p. 52), Kastle and Elvove (1909 p. 622), Kendall and Day (1911–12 p. 96), Kinyoun and Deiter (1912 p. 979), Committee A.P.H.A. (1913 p. 133), (1917 p. 97), (1920 p. 97), (1923 p. 96), (1925 p. 98), Robinson and Rettger (1916 p. 367), Roddy (1917 p. 43), Krumwiede, Kohn, Kuttner and Schumm (1918 p. 286), Tanner (1919 p. 53), Ball (1919 p. 82), Bezançon (1920 p. 342), Giltner (1921 pp. 384, 385, 387), Levine (1921 pp. 112, 113, 115), Abbott (1921 p. 524), Pitfield (1922 p. 120), Klimmer (1923 p. 209), Stitt (1923 p. 48), Park, Williams and Krumwiede (1924 pp. 127, 131).

1760. Hirschbruck and Schwer's Crystal Violet Litmus Lactose Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	20.0 g.
3. Meat extract, Liebig's	10.0 g.
4. Peptone	10.0 g.
5. NaCl	5.0 g.
6. Litmus solution, Kubel and Tiemann	130.0 cc.
7. Crystal violet B (Höchst) 0.1%	10.0 cc.

Preparation:

(1) Boil 2, 3, 4 and 5 in 1 for 1.5 hour.

(2) Filter and boil again for 30 minutes.

(3) Add 15.0 g. lactose to (2) and boil for 15 minutes

(4) Add a sterile soda solution (any desired strength, but not too strong) until red litmus paper is turned blue.

(5) Add 130.0 cc. of Kubel-Tiemann's litmus solution, and 10.0 cc. of a crystal violet solution that has been prepared by dissolving 0.1 g. Höchst crystal violet in hot sterile distilled water to (4).

(6) Mix (5) thoroly.

(7) Pour into sterile plates, about 8.0 cc. per plate, and allow to solidify with cover removed.

Sterilization: Not specified.

Use: Diagnosis of cholera. Authors reported that by transmitted and reflected light after 10 hours the cholera colonies were blue, and about 1.5 mm. in diameter. The blue color of cholera colonies was much darker than that of typhoid colonies. After 20 hours the colonies were about 2 mm. in diameter. By reflected light they appeared milk blue, and by transmitted light a deep sky blue. The entire plate remained intensively blue with a pure culture. *Bact. coli* colonies, both by transmitted and reflected light, were red.

Reference: Hirschbruch and Schwer (1903 p. 587).

1761. Chesney's Indicator Lactose Agar

Constituents:

1. Beef extract agar (3.0%)	1000.0 cc.
2. Lactose	10.0 g.
3. Brom-cresol purple (0.04%)	50.0 to 80.0 cc.

Preparation:

(1) Exact method of preparation or composition of 3.0% beef extract agar not given.

(2) Adjust (1) to pH of 7.2 to 7.4.

(3) Add 1.0% lactose from a sterile 20.0% solution to sterile (2).

(4) Add from 5.0 to 8.0 cc. of a sterile 0.05% aqueous solution of brom cresol purple, to every 100.0 cc. of sterile (3).

(5) Pour into sterile Petri dishes, about 20.0 cc. to each plate.

Sterilization: Method of sterilization of agar or lactose solution not given. Sterilize the 0.04% brom cresol purple in the autoclave.

Use: Isolation of typhoid and dysentery bacilli from stools. Author reported that medium was clear and deep blue in color. Lactose fermenters produced greenish yellow colonies with a yellow zone. Non-lactose fermenters produced bluish colonies. Typhoid colonies have typical woolly appearance. In thickly seeded plates, typhoid colonies were bluish green, while colon colonies were brilliant yellow. The addition of brilliant green did not interfere with the color production of the non-lactose fermenters.

Variants: The author prepared a similar medium as follows:

(1) Prepare a 3.0% beef extract agar and adjust to pH = 7.6 to 7.8.
(2) Clear (1) with white of an egg.
(3) Sterilization not mentioned.
(4) Add 1.0% lactose from a sterile 20.0% solution.
(5) Add for every 100.0 cc. of the medium, 10.0 cc. of an 0.04% aqueous solution of phenol red. This solution may be sterilized by autoclaving.
(6) Pour into sterile Petri dishes, about 20.0 cc. of agar to the plate.

The author reported that the medium was originally salmon pink or old rose. Lactose fermenters produced vivid greenish yellow colonies with a surrounding zone of green. The typhoid bacillus, the paratyphoid bacillus (A and B) and the dysentery bacilli (Shiga, Flexner and Hiss Y) all produced pink colonies. In very thickly seeded plates the colon colonies assumed a bright green or yellow green color and are opaque, whereas the typhoid colonies were more translucent and possessed a bluish green color. Typhoid colonies had a corrugated, woolly appearance. The addition of brilliant green did not interfere with the color production of the non-lactose fermenters.

Reference: Chesney (1922 pp. 183, 184).

1762. Salomonsen's Sucrose Extract Agar (Besson)

Constituents:

1. Water	1000.0 cc.
2. Meat extract (Liebig's)	5.0 g.
3. Peptone	30.0 g.
4. NaCl	5.0 g.
5. Agar	20.0 g.
6. Sucrose	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4 in 1.
(2) Soak 20.0 g. of chopped thread agar in cold water for several hours. Squeeze water thru a cloth.
(3) Heat (2) in (1) at 100°C. until the agar is dissolved.
(4) Readjust the reaction if necessary.
(5) Allow to cool to 55 or 60°C.
(6) Beat the white of an egg in 100.0 cc. of water and add to (5).
(7) Mix well.
(8) Autoclave at 120°C. for one hour.
(9) Add 5.0 g. of sucrose to (8).
(10) Tube.

Sterilization: Sterilize at 115°C. for 20 minutes.

Use: General culture medium.

Reference: Besson (1920 p. 43).

1763. Hesse's Starch Extract Agar (Stokes and Hachtel)

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	5.5 g.
3. Beef extract (Liebig's)	5.0 g.
4. Peptone (Witte's)	10.0 g.
5. Starch	10.0 g.
6. NaCl	8.5 g.
7. Azolitmin solution (Kahlbaum's)	

Preparation:

(1) Dry agar at 105°C. for 30 minutes.
(2) Dissolve 5.5 g. of (1) in 500.0 cc. distilled water.
(3) Add 5.0 g. beef extract to 500.0 cc. of distilled water and dissolve by heat.
(4) Filter (3) into a sterile flask and inoculate with the colon bacillus and incubate at 37°C. for 24 hours.
(5) Filter the sugar free bouillon and dissolve 10.0 g. Witte's peptone, 10.0 g. starch and 8.5 g. NaCl in it.
(6) Make up the loss due to evaporation by the addition of distilled water.
(7) Mix (6) and (2) and boil for 30 minutes, making up the loss in weight by the addition of distilled water.
(8) Filter.
(9) Adjust the reaction to neutral.
(10) Color with Kahlbaum's azolitmin solution.
(11) Distribute in 10.0 cc. lots.
(12) Store in the ice box until ready for use.

Sterilization: Autoclave at 16 pounds pressure for 20 minutes.

Use: Differentiation of typhoid bacillus, dysentery bacillus, cholera spirillum and other intestinal forms.

Reference: Stokes and Hachtel (1913 p. 348).

1764. Committee S.A.B. Starch Extract Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Water sol. starch	2.0 g.
3. Beef extract	3.0 g.
4. Peptone	5.0 g.
5. Agar	15.0 g.

Preparation:

(1) Prepare beef extract agar according to Committee A.P.H.A. (1916–1917). (See extract agar 1694). May be clarified with white of egg.

(2) Adjust to pH = 6.6 to 7.4.

(3) Add starch.

Sterilization: Method not given.

Use: Standard culture medium.

References: Committee S. A. B. (1918 p. 116), (1923 p. 10), Ball (1919 p. 78).

1765. Coplin and Bevan's Glycerol Extract Agar

Constituents:

1. Water	1000.0 cc.
2. Meat extract	5.0 g.
3. Peptone (albumin)	10.0 g.
4. Glycerol	62.0 g.
5. Agar (1.5%)	15.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Clear and filter in the same way as other media. (Method not given).

Sterilization: Not specified.

Use: Cultivation of *Micrococcus pyogenes aureus*. Authors reported that 3 or 4 days after inoculation the medium became opaque, and after 5 or 6 days the medium appeared as if tapioca was mixed with it.

Reference: Coplin and Bevan (1892 p. 70).

1766. Piettre and de Souza's Citric Acid Extract Agar

Constituents:

1. Water	1000.0 cc.
2. Meat extract	0.7 g.
3. Peptone (Chapoteaut)	10.0 g.
4. NaCl	5.0 g.
5. Citric acid	5.0 g.
6. Agar	18 to 20.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1 by heating on the salt water bath.

(2) Neutralize to phenolphthalein using 10.0% soda.

(3) Add 18.0 to 20.0 g. of agar to boiling (2).

(4) Clarify (Method not given).

(5) Tube in about 10.0 cc. lots.

(6) Add by means of a sterile pipette graduated in tenths, 0.5 cc. of a 10.0% sterile citric acid solution to each tube of melted sterile (5) cooled to 50°C.

(7) Mix thoroly and slant.

Sterilization: Method not specified.

Use: Isolation of molds and fungi, Mucor, Rhizopus, Penicillium, Aspergillus and Scelerotinia. Authors reported that it may be necessary to add 8 or even 10.0 g. of citric acid to keep down bacterial growth.

Reference: Piettre and de Souza (1922 p. 336).

1767. Fawcus' Dye Bile Salt Agar (Bezançon)

Constituents:

1. Water	900.0 cc.
2. Agar	30.0 g.
3. Meat extract, Liebig's	20.0 g.
4. Peptone (10.0% soln.)	100.0 cc.
5. Brilliant green (1:1000)	10.0 cc.
6. Picric acid (1:100)	10.0 cc.
7. NaCl	20.0 g.
8. Sodium taurocholate	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 7 and 8 in 1.

(2) Make alkaline (Indicator not specified).

(3) To 1.5 liters of agar add 10.0 cc. of 1–1000 brilliant green and 10.0 cc. of a 1–100 picric acid solution.

Sterilization: Method not given.

Use: Cultivation of colon typhoid group.

Reference: Bezançon (1920 p. 345).

1768. Percival's Urea Extract Agar

Constituents:

1. Water	1000.0 cc.
2. Beef extract (Lemco's)	5.0 g.
3. Peptone (Witte's)	10.0 g.
4. Agar	15.0 g.
5. Urea	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) If acid, neutralize with $(NH_4)_2CO_3$.

Sterilization: Sterilize in the usual manner. (Method not given).

Use: Cultivation of organisms using urea. *Urobacillus Pasteurii.* Author reported that urea fermenting colonies were surrounded with small white crystals of calcium carbonate.

Reference: Percival (1920 p. 224).

1769. Gaehtegen's Caffeine Endo Agar

Constituents:

1. Water	1000.0 cc.
2. Meat extract (Liebig's)	20.0 g.
3. Peptone (Witte siccum)	20.0 g.
4. NaCl	10.0 g.
5. Agar	80.0 g.
6. Lactose	20.0 g.
7. Fuchsin (10.0% alcoholic solution	10.0 cc.
8. Sodium sulphite (in 50.0 cc. water)	5.0 g.
9. Caffeine	6.6 g.

Preparation:

(1) Add 2, 3, 4 and 5 to 1.

(2) Place in the autoclave at 110°C. for two hours.

(3) When agar is completely dissolved, filter thru cotton.

(4) Add N/1 NaOH until it is 1.5% alkaline to phenolphthalein.

(5) Add 20.0 g. lactose, 10.0 cc. of a 10.0% alcoholic fuchsin solution and a sodium sulphite solution (5.0 g. of sodium sulphite in 50.0 cc. water).

(6) Dissolve 0.33% chemically pure crystalline caffeine in (5).

(7) Pour into sterile Petri dishes.

Sterilization: Not specified.

Use: Diagnosis of typhoid fever. After 24 hours transparent typhoid colonies appeared about 1 to 1.5 mm. in diameter. After 30 hours the colonies were 2 to 3 mm. in diameter, round, plate form, completely colorless, by transmitted light, a delicate pink in reflected light. *B. coli* colonies, if any, were red. Caffeine inhibited *B. coli.*

Reference: Gaehtegens (1905 p. 636).

1770. Wilson and Darling's Brilliant Green Bile Salt Agar

Constituents:

1. Water	1000.0 cc.
2. Lemco	10.0 g.
3. Agar	30.0 g.
4. Peptone	20.0 g.
5. Sodium taurocholate	5.0 g.
6. NaCl	5.0 g.
7. Lactose	5.0 g.
8. Brilliant green (1.0% solution)	4.0 cc.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

(2) Adjust the reaction to a +10 (Eyre)

(3) Distribute in 100.0 cc. quantities.

(4) Add 0.4 cc. of a 1.0% solution of brilliant green to each 100.0 cc. of melted medium, cooled to 50°C. when ready to pour in plates.

Sterilization: Not specified.

Use: Cultivation of colon typhoid group. Authors reported that the reaction must be +10 (Eyre). If more acid *B. coli* will grow. If more alkaline *B. typhosus* is inhibited. Some *B. coli* may grow. These produce acid and allow for the development of others.

Reference: Wilson and Darling (1918 p. 105).

1771. Olszewski and Köhler's Endo Bile Salt Agar

Constituents:

1. Water	2000.0 cc.
2. Agar	60.0 g.
3. Meat extract	20.0 g.
4. Peptone	20.0 g.
5. NaCl	10.0 g.
6. Sodium taurocholate	2.0 g.
7. Lactose	20.0 g.
8. Fuchsin (alcoholic)	10.0 cc.
9. Sodium sulphite (10.0%)	50.0 cc.

Preparation:

(1) Soak 60.0 g. agar over night in 1000.0 cc. of water.

(2) Boil for two to two and a half hours.

(3) Add 3, 4 and 5 to 1000.0 cc. of water and boil an hour.

(4) Filter.

(5) Mix (4) and (2).

(6) Neutralize with 10.0% soda solution.
(7) Boil one hour.
(8) Filter and sterilize. (Method not given).
(9) Add 10.0 cc. of 10.0% soda solution and 1.0 g. of sodium taurocholate to one liter of (8).
(10) Heat at 100°C. in the steamer for 20 minutes.
(11) Add 10.0 g. lactose, 5.0 cc. of a saturated alcoholic solution of fuchsin, and 25.0 cc. of a 10.0% sodium sulphite solution to each liter of (10).
(12) Distribute in small flasks.

Sterilization: Method of sterilization not specified.

Use: Water analysis. Enrichment medium for *Bacterium coli*.

Reference: Olszewski and Köhler (1923 p. 3).

1772. Bacto Saccharose-Mannitol Agar (Dehydrated)

Constituents:

1. Distilled water		
2. Beef extract (Bacto)	3.0	g.
3. Peptone (Bacto	5.0	g.
4. Agar (Bacto)	25.0	g.
5. Sucrose (Bacto)	10.0	g.
6. Mannitol (Bacto)	1.0	g.
7. Andrade Indicator (Difco)	0.0275	g.

Preparation:
(1) Dissolve 44.0 g. of Bacto Saccharose-Mannitol Agar (Dehydrated) in 1000.0 cc. of distilled water by boiling or autoclaving, preferably the latter.
(2) Tube.
(3) Final pH = 7.5±.

Sterilization: Sterilize in the usual manner, avoiding excessive heat.

Use: Differential tube medium.

Reference: Digestive Ferments Co. (1925 p. 12).

1773. Hesse's Lactose Glycerol Agar (Stokes and Hachtel)

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	6.0 g.
3. Beef extract (Liebig's)	5.0 g.
4. Peptone (Witte's)	10.0 g.
5. NaCl	8.5 g.
6. Lactose (1.0%)	10.0 g.
7. Glycerol (5.0%)	50.0 g.
8. Azolitmin	

Preparation:
(1) Dry agar at 150°C. for 30 minutes.
(2) Dissolve 6.0 g. of (1) in 500.0 cc. distilled water by boiling and make up the loss in weight by adding distilled water.
(3) Dissolve 3, 4, 5, 6 and 7 in 500.0 cc. of distilled water.
(4) Mix (3) and (2) and boil 30 minutes.
(5) Make up the loss in weight by the addition of distilled water.
(6) Filter.
(7) Adjust the reaction to neutrality (indicator not specified).
(8) Add sufficient azolitmin to give the desired color.
(9) Tube in 9.0 cc. lots.

Sterilization: Autoclave at 15 pounds pressure for 20 minutes.

Use: Isolation of typhoid bacillus and cholera spirillum. The author reported that typhoid colonies were pink. They had an opaque nucleus surrounded by a translucent area. *Bacillus alcaligenes* formed a blue concentric colony. Other motile organisms produced a blue colony.

Variants: The authors prepared a similar medium as follows:
(1) Dry agar at 150°C. for 30 minutes.
(2) Dissolve 5.5 g. of (1) in 500.0 cc. of distilled water.
(3) Add 5.0 g. beef extract to 500.0 cc. of distilled water.
(4) Filter (3) into a sterile flask and inoculate with the colon bacillus and incubate at 37°C. for 24 hours.
(5) Filter the sugar-free bouillon and dissolve 10.0 g. Witte's peptone, 10.0 g. lactose and 8.5 g. NaCl in it.
(6) Make up the loss due to evaporation by addition of distilled water.
(7) Mix (6) and (2) and boil for 30 minutes, making up the loss in weight by the addition of distilled water.
(8) Filter.
(9) Adjust the reaction to neutral.
(10) Add 50.0 cc. of glycerol.
(11) Color with Kahlbaum's azolitmin solution.
(12) Distribute in 10.0 cc. lots.
(13) Autoclave at 16 lbs. pressure for 20 minutes.
(14) Store in the ice box until ready for use.

Dilutions of a bile culture of a typhoid suspect was incubated for 24 hours and a series of dilutions made. These were then plated on the medium and incubated for 24 hours. Typhoid bacillus or paratyphoid bacillus colonies were medium sized, with concentric rings and showed a distinct pink color. The pink color enabled one to distinguish them from *Bacillus typhosus* or *Bacillus alcaligenes*. Colon colonies were small, moist and concentrated. The spirillum of cholera and Nassik spirillum formed a blue colony. Colonies were bluish, concentric with an opaque nucleus-like center and varying diameters from 5 to 15 millimeters.

Reference: Stokes and Hachtel (1909 p. 41), (1913 p. 346).

1774. Schnürer's Saponin Glycerol Agar

Constituents:

1. Beef extract agar (3.0%)	1000.0 cc.
2. Glycerol	30.0 cc.
3. Saponin (Merck's)	10.0 to 80.0 g.

Preparation:

(1) Prepare a beef extract agar 3.0% (contains 1.0% peptone and 0.5% NaCl).

(2) Add 3.0% glycerol and 1.0 to 8.0% saponin to (1).

Sterilization: Not specified.

Use: To study acid fastness of tubercle bacilli and timothy bacilli. Author reported that acid fastness was not changed by the presence of saponin in medium.

Reference: Schnürer (1922–23 p. 15).

1775. Bacto Russell Double Sugar Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Peptone (Bacto)	10.0 g.
3. Agar (Bacto)	15.0 g.
4. Lactose (Bacto)	10.0 g.
5. Glucose (Bacto)	1.0 g.
6. NaCl	5.0 g.
7. Andrade Indicator	0.025 g.

Preparation:

(1) Dissolve 42.0 g. of Bacto Russell Double Sugar Agar (Dehydrated) in 1000.0 cc. distilled water by boiling or autoclaving. Avoid excess heat.

(2) Tube.

Sterilization: Sterilize in the usual manner.

Use: Differentiation of *Esch. coli*, *S. paratyphi*, *S. schotmuelleri*, *Ebert typhi* and *Ebert dysenteriae*.

Reference: Digestive Ferments Co. (1925 p. 12).

1776. Nichols and Woods' Russel's Double Sugar Agar

Constituents:

1. Extract agar (3.0%)	1000.0 cc.
2. Lactose (1.0%)	10.0 g.
3. Glucose (0.1%)	1.0 g.
4. Phenol red (0.02% soln.) (5.0%)	50.0 cc.

Preparation:

(1) Prepare extract agar containing 3.0% shred agar.

(2) Clear (1).

(3) Add 1.0% lactose 0.1% glucose and 5.0% of a 0.02% watery solution of phenol red.

(4) Correct the reaction to pH 7.2–7.4 hot.

Sterilization: Not specified.

Use: To study fermentation or respiration by the typhoid bacillus.

Reference: Nichols and Wood (1922 p. 322).

1777. Bailey and Lacey's Phenol Red Lead Acetate Agar

Constituents:

1. Tap water	1000.0 cc.
2. Beef extract (Bacto)	5.0 g.
3. Peptone (P. D.)	10.0 g.
4. NaCl (B. & A.)	5.0 g.
5. Agar	15.0 g.
6. Lactose	10.0 g.
7. Glucose	1.0 g.
8. Phenol red (0.02%)	50.0 cc.
9. Lead acetate	0.5 g.

Preparation:

(1) Wash the agar in running water.

(2) Heat in 1000.0 cc. tap water until dissolved.

(3) Dissolve 2, 3 and 4 in (2).

(4) Adjust to pH = 7.4.

(5) Boil for 5 to 8 minutes.

(6) Readjust the reaction to pH = 7.4.

(7) Allow to stand and decant the clear supernatant agar.

(8) Cool to 50°C. (to prevent flocculation) and add 6, 7, 8 and 9.

(9) Tube.

Sterilization: Sterilize in the autoclave by heating at 15 pounds pressure for 15 minutes.

Use: Differentiation of colon-typhoid-dysentery group.

Reference: Bailey and Lacy (1927 p. 185).

1778. Holt, Harris and Teague's Eosine Methylene Blue Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. Peptone (Witte's)	10.0 g.
4. NaCl	5.0 g.
5. Beef extract (Liebig's)	5.0 g.
6. Sucrose	5.0 g.
7. Lactose	5.0 g.
8. Eosin (2.0% yellowish)	20.0 cc.
9. Methylene blue (0.5%)	20.0 cc.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Clear with egg white.
(3) Flask.
(4) Adjust sterile (3) to +0.8.
(5) To melted (4) add 6 and 7 and heat for 10 minutes in the Arnold.
(6) To every 50.0 cc. of the medium add 1.0 cc. of 2.0% yellowish eosin in distilled water and then 1.0 cc. of 0.5% methylene blue in distilled water
(7) Mix thoroughly, and pour in sterile Petri dishes.
(8) Dry the surface in usual way and inoculate.

Sterilization: Sterilize in Arnold on three successive days.

Use: Isolation of *Bacillus typhosus* from stools. Author reported that typhoid colonies were colorless and transparent. Colon colonies were black and did not transmit light. Sugar-free infusion may be substituted for Liebig's meat extract.

References: Holt-Harris and Teague (1916 p. 597), Stitt (1923 p. 49), Park, Williams and Krumwiede (1924 p. 128).

1778a. Krumwiede, Pratt and McWilliams Brilliant Green Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. Salt (NaCl)	5.0 g.
4. Peptone (Witte)	10.0 g.
5. Beef extract (Liebig's)	3.0 g.
6. Lactose	10.0 g.
7. Glucose	1.0 g.
8. Brilliant green (0.1% solution)	2.0 or 3.0 cc.
9. Andrade Indicator	10.0 cc.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1 in the autoclave.
(2) Adjust so that the reaction is slightly alkaline to litmus.
(3) Distribute in 100.0 cc. lots.
(4) Before use readjust so that the medium be set to Andrade Indicator. The reaction to phenolphthalein (hot titration) is 0.6 to 0.7% acid.
(5) Add Andrade indicator (amount not given).
(6) Add 1.0% lactose and 0.1% glucose to sterile (5), using sterile 25.0% solutions.
(7) Add appropriate amounts of 0.1% solution of brilliant green (0.2 cc. or 0.3 cc. of a 0.1% solution per 100.0 cc. agar).
(8) Pour in sterile Petri dishes.
(9) Allow plates to stand open until surface is dry. Inoculate as with Endo plates.

Sterilization: Sterilize (3) in the autoclave.

Use: Isolation of typhoid bacilli. Author reported that fecal types were restrained. Typhoid colonies were large and presented a snow flaky appearance with black background by light passing obliquely thru agar. Medium gave 36.0% positive increase over Endo agar.

Variants:

(a) Krumwiede, Kohn, Kuttner and Schumm prepared the medium as follows:
  (1) Dissolve 15.0 g. agar in 500.0 cc. of water by heating over gas stove.
  (2) Dissolve 3.0 g. of Liebig's extract of beef, 10.0 g. peptone and 5.0 g. NaCl in 500.0 cc. of 1 by heating over gas stove.
  (3) After complete solution mix (1) and (2).
  (4) Adjust to 0.6 or 0.7% acid to phenolphthalein (neutral to Andrade indicator).
  (5) Prepare Andrade indicator by adding 16.0 cc. of a N/1 NaOH

solution to 100.0 cc., 0.5% aqueous solution of acid fuchsin.
(6) Cool (4) and add beaten egg white.
(7) Boil and filter.
(8) Distribute in 100.0 cc. lots in bottles.
(9) Autoclave for 30 minutes at 15 pounds pressure.
(10) When ready for use melt (9) and add to each 100.0 cc., 1.0 cc. of (5), 1.0 g. lactose (or 5.0 cc. of 20.0% solution, sterilized in the Arnold), 0.1 g. glucose (or 5.0 cc. of a 2.0% solution, sterilized in the Arnold sterilizer), and 0.5, 0.3 or 0.2 cc. of a 0.1% stock solution in distilled water of brilliant green.

(b) Kligler prepared the medium as indicated above. The reaction was adjusted between pH 7.0 and 7.2. When adding the brilliant green (see step (7) above) add 0.25 cc. of a 1.0% solution of neutral red to each 100.0 cc. of agar.

(c) Park, Williams and Krumwiede prepared the medium as follows:
(1) Dissolve 30.0 g. of agar in 1000.0 cc. of water in the autoclave.
(2) Dissolve 6.0 g. meat extract, 10.0 g. NaCl and 20.0 g. peptone in 1000.0 cc. of water by heating in the Arnold.
(3) Mix (1) and (2).
(4) Add normal soda so that the final reaction will be neutral to Andrade indicator (pH 6.9).
(5) Boil 30 minutes.
(6) Cool and clear with egg.
(7) Filter until clear.
(8) Bottle in 100.0 cc. quantities.
(9) Autoclave.
(10) When ready for use melt the agar and to each 100.0 cc. add 1.0 cc. of Andrade indicator, 5.0 cc. of a sterile solution in distilled water of 20.0% lactose and 2.0% glucose, (1.0% lactose and 0.1% glucose) and the proper amount of a 0.1% solution of brilliant green in distilled water. (See (13)).
(11) Mix well.
(12) Pour thick plates. Use porous tops as the plates must be dry.
(13) To determine the amount of dye to add prepare four dilutions of dye: 1 to 500,000, 1 to 330,000, 1 to 250,000 and 1 to 200,000, which corresponds to 0.2, 0.3, 0.4 and 0.5 cc. of a 0.1% solution of dye to 100.0 cc. Inoculate these plates with a freshly isolated strain of *B. typhosus* or a positive stool. Choose the dilutions of dye that (1) at which the typhoid colonies are of good size and undiminished in number compared with the control plate, but many other fecal types are excluded, (2) a lower dilution where the typhoid colonies are reduced in size and number, but almost all the other flora have disappeared.

References: Krumwiede, Pratt and McWilliams (1916 p. 4), Krumwiede, Kohn, Kuttner and Schumm (1918 p. 275), Kligler (1918 p. 320), Giltner (1921 p. 366), Park, Williams and Krumwiede (1924 p. 128).

1779. Aronson's Fuchsin Sulphite Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	35.0 g.
3. Meat extract	10.0 g.
4. Peptone	10.0 g.
5. NaCl	5.0 g.
6. Na_2CO_3 (10.0%)	60.0 cc.
7. Sucrose (20.0%)	50.0 cc.
8. Glucose (20.0%)	50.0 cc.
9. Fuchsin (saturated)	4.0 cc.
10. Sodium sulfite (10.0%)	20.0 cc.

Preparation:
(1) Add 2 to 1 and allow to soak over night.
(2) Add 3, 4 and 5 to (1) and boil in streaming steam for 4 to 5 hours. Reaction slightly acid.
(3) Slant and remove the insoluble material.
(4) Distribute in 100.0 cc. lots by means of a graduated flask into sterile 200–250.0 cc. Erlenmeyer flasks.
(5) Prepare a 10.0% solution of Na_2CO_3 (siccum).
(6) Prepare a 20.0% solution of sucrose.
(7) Prepare a 20.0% solution of glucose.

(8) Prepare an alcoholic saturated solution of fuchsin by pulverizing commercial fuchsin in a mortar and adding absolute alcohol and placing in the incubator for a day shaking occasionally.

(9) Prepare a 10.0% solution of sodium sulphite. Boil several times to sterilize.

(10) To 100.0 cc. of liquid and sterile (4) add 6.0 cc. of sterile (5).

(11) Heat in streaming steam for 15 minutes. The agar becomes dark brown and turbid.

(12) Add 5.0 cc. sterile (6), 5.0 cc. sterile (7), 0.4 cc. (8) and 2.0 cc. (9) to each 100.0 cc. lot immediately after it is removed from the steamer.

(13) Slant the flasks so that the precipitate settles quickly to the bottom.

(14) Pour into plates, but leaving the settled precipitate in the flasks.

(15) Dry in the incubator or at 50°C. for 30 minutes.

(16) The plates are transparent and yellowish brown in color.

Sterilization: Sterilize (5), (6) and (7) by heating in steam for 30 minutes.

Use: Diagnosis of cholera. Author reported that cholera organisms gave large red colonies. Other organisms were inhibited. The more alkali added, the more coli colonies were inhibited.

Variants: Klimmer prepared the medium as follows:

(1) Soak 35.0 g. agar in a liter of water over night.

(2) Add 10.0 g. peptone, 5.0 g. NaCl and 10.0 g. meat extract to (1).

(3) Boil for 4 or 5 hours in the steamer.

(4) Distribute in 100.0 cc. lots in 200.0 cc. flasks.

(5) Add 6.0 cc. of a 10.0% solution of water free soda, 5.0 cc. of a 20.0% sucrose solution, 5.0 cc. of a 20.0% dextrin solution, 0.25 cc. of a saturated alcoholic fuchsin solution and 2.5 cc. of a 10.0% Na_2SO_3 solution to each 100.0 cc. of agar.

(6) Sterilization not specified.

References: Aronson (1915 p. 1028), Klimmer (1923 p. 218).

1780. Hesse's Malachite Green Agar (Klimmer)

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.
4. Meat extract	10.0 g.
5. Agar	35.0 g.
6. Na_2CO_3 (10.0% soln.)	30.0 to 40.0 cc.
7. Sucrose (20.0% soln.)	50.0 cc.
8. Dextrin (20.0% soln.)	50.0 cc.
9. Malachite green (chlorzinc salt c.p. sat. alc. soln.)	4 0 cc.
10. Na_2SO_3 (10.0% soln.)	25.0 cc.

Preparation:

(1) Soak 35.0 g. of agar in a liter of water over night.

(2) Add 2, 3 and 4 in (1).

(3) Boil for 4 or 5 hours in the steamer.

(4) Distribute in 100.0 cc. lots in 200.0 cc. flasks.

(5) Add 3.0 to 4.0 cc. of a 10.0% water free soda solution, 5.0 cc. of a 20.0% sucrose solution, 5.0 cc. of a 20.0% dextrin solution, 0.4 cc. of a concentrated alcoholic solution of malachite green (chlorzinc, double salt, crystalline, c.p.) and 2.5 cc. of a 10.0% Na_2SO_3 solution to each 100.0 cc. of agar.

Sterilization: Not specified.

Use: Cholera diagnosis. Author reported that cholera colonies were green after 24 to 48 hours.

Reference: Klimmer (1923 p. 219).

1781. Bacto Krumwiede Triple Sugar Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Peptone (Bacto)	10.0 g.
3. Agar (Bacto)	15.0 g.
4. Lactose (Bacto)	10.0 g.
5. Sucrose (Bacto)	10.0 g.
6. Glucose (Bacto)	1.0 g.
7. NaCl	5.0 g.
8. Andrade indicator	0.025 g.

Preparation:

(1) Dissolve 52.0 g. of Bacto Krumwiede Triple Sugar Agar (Dehydrated) in 1000.0 cc. distilled water by boiling or autoclaving. Avoid excess heat.

(2) Tube.

Sterilization: Sterilize in the usual manner.

Use: To detect intermediates of the colon-typhoid group.

Reference: Digestive Ferments Co. (1925 p. 12).

1782. Krumwiede and Kohn's Triple Sugar Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. Meat extract	3.0 g.
4. Peptone	10.0 g.
5. NaCl (salt)	5.0 g.
6. Lactose	10.0 g.
7. Sucrose	10.0 g.
8. Glucose	1.0 g.
9. Andrade indicator	10.0 cc.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Adjust slightly alkaline to litmus (or 0.6% acid to phenolphthalein hot titration).

(3) Distribute in 100.0 cc. lots.

(4) At the time of use, add 1.0% lactose, 1.0% saccharose and 0.1% glucose to melted sterile (3). (These amounts may be conveniently added from a stock solution containing 20.0% lactose, 20.0% saccharose and 2.0% glucose that has been sterilized intermittently. Five cc.'s of this solution gives the desired concentration.

(5) Add 1.0% Andrade indicator and adjust reaction. The medium is to be red when hot and colorless when cold. (If it is not red when warm the medium is too alkaline, and if it fails to decolorize when cool it is too acid.)

(6) Tube from a sterile covered funnel, preferably in narrow tubes.

(7) Steam for 20 minutes.

(8) Slant leaving a generous butt for inoculation.

(9) Incubate over night to test sterility.

Sterilization: Sterilize (3) in the autoclave.

Use: Isolation of para-typhoid bacilli. Used especially to isolate the intermediates of the colon-typhoid group.

References: Krumwiede and Kohn (1917 p. 226), Park, Williams and Krumwiede (1924 p. 128).

1783. Amoss' Four Sugar Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Beef extract	3.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Agar	15.0 g.
6. Lactose	2.5 g.
7. Raffinose	2.5 g.
8. Sucrose	2.5 g.
9. Salicin	2.5 g.
10. Lead acetate (0.25% soln.)	250.0 cc.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8, 9 and 10 in 1.

(2) Adjust to pH = 7.2.

(3) Distribute in 4.0 cc. lots.

(4) Add to each tube 1.0 cc. of a 0.25% solution of lead acetate.

Sterilization: Not specified.

Use: Cultivation of *Bacillus typhi murium*. Author reported that *Bacillus typhi murium* produced browning of medium.

Reference: Amoss (1922 p. 28).

1784. Amoss' Sucrose Salicin Agar (Topley and Ayrton)

Constituents:

1. Water	1000.0 cc.
2. Beef extract (Lemco)	5.0 g.
3. Peptone (Witte)	10.0 g.
4. NaCl	5.0 g.
5. Lactose	10.0 g.
6. Sucrose	10.0 g.
7. Salicin	10.0 g.
8. Andrade indicator	10.0 cc.
9. Agar	25.0 g.
10. Lead acetate (1.0%)	50.0 cc.
11. Na_2HPO_4 (1.0%)	50.0 cc.

Preparation:

(1) Dissolve 2, 3, and 4 in 1 by heating in the steamer.

(2) Add 25.0 g. agar and steam until dissolved.

(3) Remove from the steamer and add the white of an egg.

(4) Coagulate in the steamer.

(5) Filter.
(6) Adjust to pH = 7.1.
(7) Add 10.0 cc. of Andrade's indicator.
(8) Autoclave at 107°C. for 20 minutes.
(9) Dissolve 5, 6 and 7 in the minimum quantity of distilled water (about 30 cc.).
(10) Mix sterile (9) with (8) and stir with a sterile glass rod.
(11) Distribute into small flasks or tubes.
(12) Place in the steamer for 10 minutes. pH at this point should be near pH = 7.4.
(13) Prepare a 1.0% neutral lead acetate (lead diacetate) by rapidly raising 30.0 cc. of sterile distilled water to a boil, and adding 0.3 g. of neutral lead acetate (lead diacetate).
(14) Add (13) to (11) using a sterile pipette in the proportion of 5.0 cc. of (13) to each 100.0 cc. of (11).
(15) Shake the flask quickly and vigorously.
(16) Add with a sterile pipette, 5.0 cc. of a 1.0% solution, Na_2HPO_4 for each 100.0 cc. of medium.
(17) Shake again.
(18) Place in the water bath at 55°C.
(19) Distribute into tubes.

Sterilization: Sterilize (9) by placing in the steamer for 20 minutes. See step (8) for sterilization of agar. Final sterilization of tubed medium not specified.

Use: Enumeration of *B. aertryke* in feces. The authors reported that 1.0% glucose gave better results than lactose, sucrose and salicin, if *B. aertryke* outnumber the other organisms in the feces.

Reference: Topley and Ayrton (1923–24 p. 230).

1785. Frost's Glucose Agar

Constituents:

1. Nutrient agar	1000.0 cc.
2. Glucose (1.0%)	10.0 g.

Preparation:
(1) Add 1.0% glucose to nutrient agar.
(2) Tube.

Sterilization: Sterilize in the steamer.

Use: General culture medium.

Variants:
(a) Heinemann added 1.5% glucose to nutrient agar and sterilized the medium in the autoclave at 120°C. for 5 minutes.
(b) Löhnis added 0.5% glucose, dissolved in a little water to sterile melted nutrient agar.
(c) Lignieres added 0.25% agar and 0.25% glucose to peptone bouillon and sterilized at 120°C. for 15 minutes.
(d) Giltner added 1.0% glucose and 1.0% $CaCO_3$ to hot agar, and sterilized by the discontinuous method.
(e) Harvey added sufficient sterilized precipitated chalk to render glucose-agar white and opaque.
(f) Puder cultivated *Rhabditis pellio*, a nematode, on a medium prepared as follows:
  (1) Prepare bouillon. Reaction to be alkaline.
  (2) Mix 10.0 cc. of (1) with 90.0 cc. water.
  (3) Dissolve 1.5 g. of agar in (2).
  (4) Add 2.0 g. of glucose and 8 drops of concentrated (strength not given) alkaline to (3).
  (5) Sterilize on three successive days for 30 minutes each day in a steamer.
  (6) Pour into sterile Petri dishes.

References: Frost (1903 p. 64), Heinemann (1905 p. 20), Löhnis (1913 p. 17), Lignieres (1919 p. 1091), Giltner (1921 p. 365), Harvey (1921–22 p. 89), Puder (1923 p. 99), Stitt (1923 p. 38).

1786. Mankowaki's Indigo Carmine Glucose Agar

Constituents:
1. 0.33 to 0.5% glucose agar.
2. Fuchsin (acid, sat. solution in 1.0% KOH).
3. Indigo carmine (sat. aqueous solution).

Preparation:
(1) Prepare a 0.33 to 0.5% glucose agar. Reaction to be neutral.
(2) Prepare a saturated solution of acid fuchsin in a 1.0% KOH solution. (Acid fuchsin may be added to a 1.0% KOH solution until a dark black brown color is reached.)
(3) Prepare a watery saturated solution of indigo carmine.
(4) Add 2.0 cc. of (2) and 1 0 cc. of (3) to 22.0 cc. of distilled water. This solution is dark blue and reaction slightly alkaline.

(5) Add (4), drop by drop, to (1) until the medium is colored blue and then violet blue.
(6) Distribute into test tubes.
(7) Add to each test tube a drop of a watery saturated solution of indigo carmine.

Sterilization: Not specified.

Use: Differentiation between typhoid bacilli and *Bacterium coli*. Author reported that typhoid bacilli colored the blue medium a raspberry or carmine red color. *Bacterium coli* colored the medium bluish green and finally decolorized it.

Reference: Mankowaki (1900 p. 22).

1787. Rivas' Glucose Agar

Same as medium 924, but solidified with agar.

1788. Hall and Ellefson's Gentian Violet Glucose Agar

Constituents:

1. Glucose agar	1000.0 cc.
2. Gentian violet	1:100,000

Preparation:

(1) Prepare glucose agar in sufficient quantity.
(2) Add gentian violet so that it be present in 1:100,000 dilution.

Sterilization: Not specified.

Use: Cultivation of gram positive sporulating anaerobes.

Variants: Authors used 1:1000 and 1:10,000 of gentian violet, but best results were obtained with 1:100,000 concentration of gentian violet.

Reference: Hall and Ellefson (1918 p. 336).

1789. Wilson and Blair's Sulphite Glucose Agar

Constituents:

1. Glucose agar (3.0%)	100.0 cc.
2. Ferric chloride (8.0% soln.)	1.0 cc.
3. Na_2SO_3 (20.0%)	10.0 cc.

Preparation:

(1) Add 1.0 cc. of an 8.0% ferric chloride solution, 0.6 cc. of a 10.0% sodium hydrate solution and 10.0 cc. of a 20.0% anhydrous sodium sulphite solution to freshly boiled melted nutrient glucose agar containing 3.0% agar.
(2) Add 40.0 cc. of (1) to 40.0 cc. of water.
(3) Pour into plates.
(4) When the medium has solidified cover the surface with a layer of (1) mixed with an equal volume of sterile water.

Sterilization: Not specified.

Use: To study reduction of sulphite in water analysis. Author reported that a pure water showed no black colonies using 40.0 cc. of the water as an inoculum, a potable water showed not more than 4, a sand-filtered water showed not more than one. The author used a similar medium for the enrichment of typhoid bacilli and detection of sulphite reducers in water.

Variants: The author prepared a similar medium as follows:

(1) Add 1.0 cc. of an 8.0% ferric chloride solution, 0.6 cc. of a 10.0% sodium hydrate solution and 10.0 cc. of a 20.0% anhydrous Na_2SO_3 solution to freshly boiled melted nutrient glucose agar containing 3.0% agar.
(2) Add from 1 to 1000, to 1 to 20,000 brilliant green to (1).
(3) Add 40.0 cc. of (2) to 40.0 cc. of water under investigation.
(4) Pour into plates.
(5) When the medium has solidified cover the surface with a layer of (1) mixed with an equal volume of sterile water.

Reference: Wilson and Blair (1925 p. 112).

1790. Scheffler's Neutral Red Glucose Agar

Constituents:

1. Nutrient agar	1000.0 cc.
2. Glucose	3.0 or 10.0 g.
3. Neutral red (concentrated solution)	10.0, 5.0 or 2.5 cc.

Preparation:

(1) Prepare a nutrient agar.
(2) Add 0.3 or 1.0% dextrose to (1).
(3) Add varying amounts of a concentrated solution of neutral red to 100.0 cc. of (2). Amounts used were 1.0, 0.5 or 0.25 cc.

Sterilization: Not specified.

Use: Detection of *Bact. coli*. Author reported that all coli strains investigated gave a fluorescence in a greater or less degree after 48 hours. Fluorescence may usually be obtained after 24 hours using

0.3% glucose and 1.0 cc. dye per 100.0 cc. agar.

Variants:

(a) Makgill added 1.0% of a saturated solution of neutral red to glucose agar.

(b) Heinemann added sufficient 0.5% neutral red to give a red color to 1.0% glucose agar.

(c) Bezançon prepared a similar medium as follows:

(1) Dissolve 5.0 g. of agar in 1000.0 cc. of bouillon.

(2) To melted (1) nearly solid, add 2.0 g. glucose and 0.7 cc. of a aqueous saturated solution of neutral red.

(3) Tube.

(4) Sterilization not specified.

(d) Rothberg and Scheffler (Klimmer) prepared a medium as follows:

(1) Add 0.3% glucose to melted sterile nutrient agar.

(2) Add 1.0 cc. of a saturated (cold) solution of neutral red sterilized in the steamer to each 100.0 cc. of (1).

(e) Cunningham prepared a medium as follows:

(1) Add 1.5% agar to bouillon.

(2) Steam for 30 minutes to dissolve the agar.

(3) Boil over an open flame for 15 minutes, stirring constantly.

(4) Adjust the reaction to a slight alkalinity using turmeric paper as an indicator (distinctly brown).

(5) Filter while hot thru a plug of clean cotton-wool in the bottom of an enamelled funnel.

(6) Add 0.5% glucose dissolved in a few cc. of water.

(7) Stir to thoroly mix.

(8) Tube.

(9) Sterilize intermittently in steam.

References: Scheffler (1900 p. 202), Magkill (1901 p. 431), Heinemann (1905 p. 127), Tanner (1919 p. 50), Bezançon (1920 p. 114), Klimmer (1923 p. 211), Cunningham (1924 p. 16), Savage (1901 p. 437), Irons (1902 p. 315).

1791. Martin and Loiseau's Glucose Litmus Agar

Constituents:

1. Distilled water	200.0 cc.
2. Glucose	3.0 g.
3. Litmus (soln.)	30.0 cc.
4. Nutrient agar (3.0%)	

Preparation:

(1) Dissolve 3.0 g. of glucose and 30.0 cc. of litmus in 200.0 cc. distilled water.

(2) Add five parts sterile (1) to six parts of sterile nutrient 3.0% agar in tubes. The mixture should be a violet tint.

Sterilization: Sterilize (1) by filtering. Method of sterilization of 3.0% agar not given.

Use: Differentiation of diphtheria and pseudo diphtheria. True diphtheria bacilli changed the color of the medium to red and grew only anaerobically. Pseudodiphtheria strains did not change the color and grew only on the surface.

Reference: Martin and Loiseau (1919 p. 73). Taken from (1919 p. 185).

1792. Frost's Lactose Agar

Constituents:

1. Nutrient agar	1000.0 cc.
2. Lactose (1.0%)	10.0 g.

Preparation:

(1) Add 1.0% lactose to nutrient agar.

(2) Tube.

Sterilization: Sterilize in the steamer.

Use: General culture medium.

Reference: Frost (1903 p. 64).

1793. Wurtz's Litmus Lactose Agar

Constituents:

1. Infusion agar	1000.0 cc.
2. Lactose (2.0%)	20.0 g.
3. Litmus	

Preparation:

(1) Add 2.0% lactose to sterile infusion agar.

(2) Tube.

(3) Melt sterile (2) and add sufficient tincture of litmus to give a violet color.

(4) Pour sterile (3) into plates.

Sterilization: Method of sterilization of (1) or (2) not given. Sterilize (3) at 100°C.

Use: Differentiation of *Bact. coli* and Eberth's bacillus. Author reported that *Bact. coli* fermented lactose with the production of acid. Typhoid bacillus colonies were colorless, colon colonies were red.

Variants:

(a) Smith specified that the reaction of the agar be slightly alkaline (0.5%).

(b) Smith (1905) also gave the following method of preparation:

- (1) Add 10.0 g. of c.p. lactose to 1000.0 cc. of sugar-free meat infusion agar.
- (2) Add 20.0 cc. of a saturated watery solution of lime-free blue litmus to (1).
- (3) Sterilization not specified.

(c) Heinemann prepared a similar medium as follows:

- (1) Add 1.0% lactose to sugar-free infusion agar.
- (2) Tube in 8.0 cc. quantities.
- (3) Add 1.0 cc. of 1.0% sterile litmus solution to each tube before using.

(d) Committee A. P. H. A. (1913).

- (1) Boil 10.0 or 15.0 g. of thread agar in 500.0 cc. of water for half an hour and make up weight to 500.0 g. or digest for 15 minutes in the autoclave. Cool to 60°C.
- (2) Infuse 500.0 g. of lean meat for 24 hours with 500.0 cc. distilled water in a refrigerator.
- (3) Make up lost weight.
- (4) Strain thru cotton flannel.
- (5) Weigh.
- (6) Add 2.0% Witte's peptone and warm on the water bath until solution is complete. Do not heat above 60°C.
- (7) Mix 500.0 cc. (6) and 500.0 cc. of (1), keeping the temperature below 60°C.
- (8) Titrate and adjust the reaction to neutral to phenolphthalein, adding normal HCl or NaOH.
- (9) Heat on a water bath for 40 minutes.
- (10) Make up lost weight.
- (11) Readjust to neutrality if necessary and boil 5 minutes.
- (12) Restore lost weight.
- (13) Filter thru absorbent cotton and cotton flannel.
- (14) Titrate and record final reaction.
- (15) Add 1.0% lactose and sufficient azolitmin solution.
- (16) Tube in 10.0 cc. quantities.
- (17) Sterilize for 15 minutes in the autoclave at 120°C., or for 30 minutes on each of 3 successive days.

(e) Ball (1919) prepared a similar medium as follows:

- (1) Add 1.0% lactose to nutrient agar just before sterilization.
- (2) Reaction of (1) to be neutral.
- (3) Boil 1.0% Kahlbaum's azolitmin for 5 minutes.
- (4) Add (3) to the tubes just before sterilization, or if to be used in plates, add at the time of plating.

(f) Abbott (1921) gave the following method of preparation:

- (1) Prepare a nutrient agar so that the alkalinity is such that it requires 0.1 cc. of a 1:20 normal H_2SO_4 solution to neutralize 1.0 cc. of medium. Indicator not specified.
- (2) Add 2.0 to 3.0% lactose.
- (3) Decant into test tubes.
- (4) Sterilize in the usual way (method not given).
- (5) Add sufficient sterile litmus tincture to each tube to give a decided but not intense blue color. Add the litmus under aseptic conditions.

(g) Giltner gave the following methods of preparation. The media were used in water analysis.

- (1)
  - (1) Prepare infusion agar using equal parts meat infusion and water, with 1.0% peptone and 1.5% agar.
  - (2) Adjust the reaction to +1.0.
  - (3) Add 1.0% lactose and 2.0% azolitmin solution just before tubing.
  - (4) Tube.
  - (5) Sterilize for 30 minutes on 3 successive days.
- (2)
  - (1) Preparation of meat infusion not given.
  - (2) Strain (1) thru a piece of clean cheese cloth.
  - (3) Place 2.0% washed agar in 500.0 cc. distilled water.
  - (4) Weigh (3).
  - (5) Digest over a free flame.
  - (6) Add distilled water to make up the loss in weight.

(7) Add 2.0% lactose and 2.0% peptone to hot (6) and mix until solution is complete.
(8) Add (2) to (7).
(9) Adjust the reaction to 0.
(10) Add 2.0% azolitmin solution.
(11) Boil over a free flame.
(12) Distribute in 100.00 cc. lots in 250.0 cc. Florence flasks.
(13) Sterilization not specified.

References: Wurtz (1897 p. 43), Smith (1905 p. 94), Heinemann (1905 p. 127), Committee American Public Health (1913 p. 129), Ball (1919 p. 78), Abbott (1921 p. 142), Giltner (1921 pp. 379, 380).

1794. Gassner's Metachrome Yellow Water Blue Lactose Agar (Klimmer)

Constituents:

1. Nutrient agar	2000.0 cc.
2. Metachrome yellow (2.0% soln.)	125.0 cc.
3. Water blue (6B extra "Afga" 1.0% soln.)	175.0 cc.
4. Lactose	100.0 g.

Preparation:

(1) Make two liters of agar prepared from yeast or meat and peptone, slightly alkaline.
(2) Add 125.0 cc. of a 2.0% solution of metachrome yellow (boiled two minutes).
(3) Dissolve 100.0 g. lactose in 175.0 cc. of a 1.0% solution of water blue (6B extra "Afga") and boil 10 minutes.
(4) Add (3) to (2).

Sterilization: Not specified.

Use: Detection of typhoid bacilli. Author reported that *B. coli* colonies were deep blue. Typhoid and dysentery colonies lightened up the medium, coloring it a greyish yellow.

Reference: Klimmer (1923 p. 216).

1795. Hirschbruch and Schwer's Azolitmin Crystal Violet Lactose Agar

Constituents:

1. Nutrient agar (1.0 to 1.5%)	1000.0 cc.
2. Lactose	15.0 g.
3. Crystal violet (0.1% soln.)	10.0 cc.
4. Azolitmin	0.4 g.

Preparation:

(1) Prepare nutrient agar containing 1.0 to 1.5% agar.
(2) Place 1.5 g. of lactose into a sterile flask.
(3) Pour approximately 100.0 cc. of the liquid agar over the lactose and mix well.
(4) Boil 100.0 g. of distilled water 15 minutes.
(5) Dissolve 0.1 g. crystal violet in (4).
(6) Add a weak soda solution that has been boiled several times to (3) until the desired degree of alkalinity is obtained.
(7) Dissolve 0.04 g. azolitmin in a little water and boil.
(8) Add 1.0 cc. of (5) and the azolitmin solution to (6) (the melted lactose agar) that has been cooled to 45°C.
(9) Mix well and after about ten minutes, pour into sterile Petri dishes. Allow the agar to solidify with the covers removed.

Sterilization: Method not given.

Use: Diagnosis of cholera. Authors reported that cholera colonies were deep blue.

Reference: Hirschbruch and Schwer (1904 p. 150).

1796. Ramond's Rubine Acid Lactose Agar

Constituents:

1. Nutrient agar	1000.0 cc.
2. Lactose	40.0 g.
3. Rubine acid	

Preparation:

(1) Add 4 parts per 100 of lactose to nutrient agar.
(2) Add several grains of rubine acid until the medium is colored red.
(3) Heat to 70 or 80°C. and add a saturated solution of Na_2CO_3 until the color disappears.
(4) Filter thru paper.
(5) Distribute in tubes.

Sterilization: Sterilize at 115°C. for five minutes.

Use: Detection of typhoid bacilli. Author reported that *Bact. coli* colonies colored the medium red. Typhoid colonies did not change the color.

References: Ramond (1896 p. 884), Wurtz (1897 p. 43).

1797. Delta's Fuchsin Lactose Agar

Constituents:

1. Distilled water	4.0 cc.

2. Agar (3.0%)	100.0 cc.
3. Lactose	1.5 g.
4. Fuchsin acid (0.5%)	10.0 cc.
5. Na_2CO_3	

Preparation:

(1) Prepare a 3.0% nutrient agar with a reaction slightly alkaline to litmus.

(2) Dissolve 1.5 g. lactose in 4.0 cc. of distilled boiling water. Boil for 30 seconds.

(3) Prepare a 0.5% solution of acid fuchsin.

(4) Boil (3) and decolorize by adding four drops of a normal Na_2CO_3 solution.

(5) Boil again until it assumes a port wine color.

(6) Add (2) and (5) to 100.0 cc. of (1).

Sterilization: Not specified.

Use: Examination of feces.

Variants: The author reported that any sugar may replace lactose. The medium may be made more differentiating by the addition of "nutrose or caffeine or malachite green (in tested solution as for Lentz and Tietz's medium) or crystal violet (10 drops of a 1 to 1000 solution). In the last two combinations the background is green (or blue) and the coli colonies are violet (or red)."

Reference: Delta (1915 p. 1053).

1798. Kindborg's Fuchsin Malachite Green Agar

Constituents:

1. Agar	1000.0 cc.
2. Lactose	14.0 g.
3. Fuchsin acid (3.0%)	50.0 cc.
4. Malachite green (1:10,000 soln.)	10.0 cc.

Preparation:

(1) Prepare nutrient agar, neutral to litmus.

(2) Prepare a 3.0% stock solution of acid fuchsin.

(3) Prepare a solution of malachite green 1:10,000 (from a 0.1:100).

(4) Dissolve 14.0 g. lactose in about 40.0 cc. water.

(5) When the agar has cleared somewhat by settling, add 50.0 cc. of the red and 10.0 cc. of the green liquid and finally the lactose solution.

(6) Pour sterile (5) into sterile plates.

Sterilization: Sterilize (5) one-half hour in the autoclave.

Use: Detection of typhoid fever and dysentery. Author reported that typhoid, paratyphoid and dysentery bacilli gave light colorless colonies. Coli forms gave small red colonies. They were inhibited. Cholera colonies were colorless but the medium was not selective for this organism.

Reference: Kindborg (1915–16 p. 445).

1799. Bitters' China Blue Malachite-green Agar (Klimmer)

Constituents:

1. Agar (2.0 or 3.0%)	1000.0 cc.
2. Lactose (2.0%)	20.0 g.
3. China blue (Höchst. sat. solution)	
4. Malachite green (Höchst. 0.1% soln.)	25.0 cc.

Preparation:

(1) Prepare a 2.0 or 3.0% nutrient agar.

(2) Neutralize to litmus by the addition of NaOH.

(3) Add 2.0% lactose.

(4) Boil several minutes.

(5) Add 9 drops of a saturated solution of China blue (Höchst.) to each 100.0 cc. of agar.

(6) Add 2.5 cc. of a 0.1% solution of Höchst. crystalline extra malachite green to each 100.0 cc. (The malachite green may be omitted.)

(7) Heat for 10 minutes in a steamer.

(8) Pour in plates.

Sterilization: Not specified.

Use: Differentiation of colon typhoid group. Author reported that coli colonies were blue; typhoid colonies yellow or colorless.

Reference: Klimmer (1923 p. 217).

1800. Liebermann and Acéls' Congo Red Agar (Klimmer)

Constituents:

1. Nutrient agar	1000.0 cc.
2. Lactose	15.0 g.
3. Congo red	3.0 g.

Preparation:

(1) Add 15.0 g. of lactose and 3.0 g. of Congo red to a liter of slightly alkaline nutrient agar.

(2) Boil.

(3) Mix thoroly.

Sterilization: Not specified.

Use: Differentiation of colon typhoid group. Author reported that typhoid colonies were red and transparent; colon colonies bluish black.

Reference: Klimmer (1923 p. 216).

1801. Massini's Triple Dye Lactose Agar

Constituents:

1. Nutrient agar (3.0%)	1000.0 cc.
2. Lactose	30.0 g.
3. Egg chrome yellow 2 G.	1.0 g.
4. Helvetia blue	0.65 g.

Preparation:

(1) Prepare nutrient 3.0% agar.

(2) Add 30.0 g. lactose, 1.0 g. egg chrome yellow 2 G. and 0.65 g. helvetia blue to 1000.0 cc. of (1).

Sterilization: Sterilize in the Arnold on each of three consecutive days for 15 minutes.

Use: Detection of typhoid and dysentery. The medium was turned blue by *B. coli* and other acid forming organisms while the typhoid-dysentery group gave a yellow color.

Reference: Massini (1918 p. 887) taken from (1918 p. 204).

1802. Lange's Starch Agar

Constituents:

1. Nutrient agar	1000.0 cc.
2. Starch (rice) (5.0%)	50.0 g.

Preparation:

(1) Add 40.0 cc. of a 10.0% soda solution to 1 liter of agar neutral to litmus.

(2) Prepare a 5.0% rice starch paste by boiling rice starch in water.

(3) Mix 6 parts sterile alkaline agar with one part sterile rice starch paste.

(4) Pour into Petri dishes.

Sterilization: Method of sterilization of agar not given. Sterilize (2) in the autoclave.

Use: Diagnosis of cholera. Author reported that cholera vibrio showed characteristic growth after from 14 to 20 hours. Also used to determine the production of diastase by bacteria.

Variants:

(a) Tanner added 2.0% paste of potato starch to melted and cooled nutrient agar.

(b) Allen added 2.0% water soluble starch to nutrient agar and sterilized the medium in the autoclave.

References: Lange (1916 p. 153), Tanner (1919 p. 60), Allen (1918 p. 15).

1803. Hoffmann's Nitrate Starch Agar

Constituents:

1. Nutrient agar	1000.0 cc
2. Starch	5.0 g.
3. KNO_3 (0.1%)	1.0 g.

Preparation:

(1) Prepare 1000.0 cc. nutrient agar.

(2) Add 0.5% starch and 0.1% KNO_3 to (1).

Sterilization: Not specified.

Use: Differential count of bacteria in soil—nitrate reduction. Treat the seeded plates with a dilute solution of KI in dilute HCl. Author reported that colonies reducing nitrate to nitrite had a blue halo around them following this treatment.

Reference: Hoffmann (1912 p. 386).

1804. Hoffmann's Nitrate Starch Agar

Same as medium 1803 but using KNO_2 instead of KNO_3. The author reported that colonies reducing nitrite produced a clear halo around the colony when a dilute KI solution in dilute HCl was added. If a yellowish halo was produced around a colony when a dilute solution of Nessler's reagent be added, ammonia was formed.

1805. Khouvine's Cellulose Agar

Constituents:

1. Nutrient agar.
2. Cellulose.

Preparation:

(1) Add very finely divided Berzelius filter paper or precipitated cellulose to nutrient agar.

Sterilization: Not specified.

Use: Isolation and enrichment of *B. cellulose dissolvens.*

Variants: The author prepared the agar as indicated:

(1) Place a layer of sterile agar in a sterile Petri dish.

(2) Add a sheet of sterile filter paper.

(3) Cover with a layer of melted agar containing the organisms.

Reference: Khouvine (1923 pp. 714, 715).

1806. Scales' Salt Cellulose Agar

Constituents:

1. Water.
2. Filter paper
3. Nutrient agar.
4. Salts.

Preparation:

(1) Dilute 100.0 cc. of concentrated H_2SO_4 with 60.0 cc. distilled water in a two liter Erlenmeyer flask.
(2) Cool to about 60 or 65°C.
(3) Moisten 5.0 g. of filter paper with water.
(4) Add (3) to (2) and shake until the paper is dissolved.
(5) When solution is complete fill the flask as quickly as possible with cold tap water. The task of dissolving the filter paper and filling the flask requires about one minute.
(6) The rapid addition of cold water precipitates the cellulose in small flocks.
(7) Throw the precipitate on a filter paper and wash with distilled water until free from H_2SO_4. This requires about 3 hours time and only 5 liters of distilled water. A 12 inch funnel with a folded filter is the best apparatus to filter with.
(8) During the washing do not allow the volume of water in the funnel to get below 100.0 cc.
(9) When the wash water is free from H_2SO_4 as shown by the addition of $BaCl_2$ solution, allow the volume in the funnel to drain to about 200.0 cc.
(10) Brush any cellulose particles clinging on the dry filter paper into the suspension.
(11) Punch a hole in the bottom of the filter, collecting the cellulose suspension.
(12) Wash the filter with a stream of water from a wash bottle.
(13) Make the suspension to 500.0 cc.
(14) Prepare 500.0 cc. of nutrient agar with salts (composition not specified).
(15) Mix (13) and (14).

Sterilization: Not specified.

Use: Cultivation of organisms capable of utilizing cellulose.

Reference: Scales (1916 p. 662).

1807. Cantani's Basal Glycerol Agar

Constituents:

1. Nutrient agar.
2. Glycerol.

Preparation:

(1) Mix equal amounts of glycerol and one of the added nutrients. Place in an Erlenmeyer flask.
(2) Melt tubes of sterile nutrient agar and add 0.5 to 0.75 cc. of sterile (1) to each tube.
(3) Incubate 24 hours to test sterility.

Sterilization: Storing the fluid in glycerol tends to sterilize it. After a time test the sterility of the mixture. Method of sterilization of agar not given.

Use: Cultivation of parasitic and pathogenic forms.

Added nutrients: The author added one of the following: Urine, pus, sperm, milk, egg white and other albuminous materials.

Reference: Cantani (1910 p. 471).

1808. Wurtz's Glycerol Agar

Constituents:

1.	Nutrient agar	1000.0 cc.
2.	Glycerol (6.0%)	60.0 g.

Preparation:

(1) Add 6.0% sterile glycerol to sterile nutrient agar under aseptic conditions.

Sterilization: Method not specified.

Use: Cultivation of tubercle bacilli. Similar media have been used to cultivate a large variety of other pathogenic forms.

Variants:

(a) Glücksmann (1897) added 7.7% glycerol to 1.5% nutrient agar and cultivated diphtheria bacilli.
(b) Thoinot and Masselin added 4.0 to 6.0% glycerol to nutrient agar.
(c) Dalton and Eyre added 5.0% glycerol to nutrient agar and adjusted the reaction to +10 on Eyre's scale. They cultivated *Micrococcus melitensis.*
(d) Smith cultivated plant parasites in a medium prepared by the addition of 50.0 cc. of Schering's c.p. twice distilled glycerol to nutrient agar.
(e) Roux and Rochaix added 1.0 to 5.0% glycerol to agar. They suggested the addition of several drops of a saturated gum arabic solution. This

favored the adherence of the agar to the walls of the containing vessels.

Reference: Wurtz (1897, p. 46), Glücksmann (1897 p. 436), Thoinot and Masselin (1902 p. 35), Smith (1902 p. 92), Dalton and Eyre (1904 p. 159), Heinemann (1905 p. 128), Smith (1905 p. 196), Roux and Rochaix (1911 p. 117), Roddy (1917 p. 43), Ball (1919 p. 74), Besson (1920 p. 43), Dopter and Sacquepee (1921 p. 128), Pitfield (1922 p. 117), Stitt (1923 p. 38), Cunningham (1924 p. 165).

1809. Scheffler's Indicator Glycerol Agar

Constituents:

1. Glycerol agar	1000.0 cc.
2. Neutral red (Concentrated aqueous soln.)	15.0 cc.

Preparation:

(1) To 100.0 cc. of glycerol agar add 1.5 cc. of a concentrated watery solution of neutral red.

Sterilization: Not specified.

Use: Detection of *Bacterium coli.* Author reported that after 24 hours in neutral red, a slight greenish coloration was formed, in 48 hours a distinct green fluorescence. At the end of 24 hours with saffarin, the under surface was lighter and after 48 hours decolorization occurred.

Variants: The author substituted a concentrated watery solution of saffarin for neutral red.

Reference: Scheffler (1900 p. 201).

1810. Mandelbaum's Rosolic Acid Glycerol Agar

Constituents:

1. Glycerol agar 6.0%.
2. Rosolic acid 1.0% alcoholic soln.

Preparation:

(1) Prepare a 6.0% glycerol agar.

(2) Prepare a 1.0% alcoholic solution of rosolic acid.

(3) Melt sterile (1), cool to 50°C. and to each tube add 0.3 cc. of (2).

Sterilization: Not specified.

Use: Differentiation of typhoid and metatyphoid bacilli. Author reported that metatyphoid bacilli gave red colonies. The typhoid colonies were yellow.

Reference: Mandelbaum (1912 p. 48).

1811. Heinemann's Litmus Mannitol Agar

Constituents:

1. Sugar free agar	1000.0 cc.
2. Mannitol (1.0%)	10.0 g.
3. Litmus	

Preparation:

(1) Add 1.0% mannite to sugar-free agar.

(2) Tube in 8.0 cc. quantities.

(3) Add 1.0 cc. of 1.0% sterile litmus solution to each tube before use.

Sterilization: Not specified.

Use: General culture medium.

Reference: Heinemann (1905 p. 127).

1812. Penfold's Phenylacetate Agar

Constituents:

1. Nutrient agar	150.0 cc.
2. Phenylacetate (10.0% soln.)	

Preparation:

(1) Dissolve phenylacetate in saturated Na_2CO_3 solution until the solution is just faintly alkaline to neutral litmus paper.

(2) Add distilled water to render the solution of 10.0% strength.

(3) Filter thru a Doulton filter.

(4) Pour ascending quantities of the solution into Petri dishes and 15.0 cc. of melted agar added to each plate.

Sterilization: Not given.

Use: To show inhibition of bacterial growth using members of the colon-aerogenes group. Author reported that *B. coli* produced papillated colonies and showed marked variability in the size of its colonies.

Reference: Penfold (1913–14 p. 38).

1813. Wurtz's Phenol Agar (Copeland)

Constituents:

1. Wurtz agar.
2. Phenol 2.0% solution.

Preparation:

(1) Method of preparation or composition of Wurtz agar not given.

(2) Add 0.2 cc. of a 2.0% solution of carbolic acid to the agar. Amount of agar not specified.

Sterilization: Not specified.

Use: Isolation of *Bacillus coli communis* from water.

Variants: Behmer's (Klimmer) added 2.0 cc. of a 5.0% phenol solution to each 100.0 cc. agar.

Reference: Copeland (1901 p. 493), Klimmer (1923 p. 227).

1814. Penfold's Monochlorhydrin Agar

Constituents:

1. Agar.
2. Monochlorhydrin 20.0% solution.

Preparation:

(1) Prepare a 20.0% solution of monochlorhydrin.

(2) Place ascending quantities of sterile (1) in Petri dishes.

(3) Add 15.0 cc. of sterile agar to each plate. Mix well.

Sterilization: Filter (1) to sterilize. Method of sterilization of agar not given.

Use: To show inhibition of bacterial growth using members of colon aerogenes group. Author reported that *B. coli* produced various sized colonies and papillae may be formed.

Reference: Penfold (1913–14 p. 39).

1815. Jacobson's Ethylcinnamic Ether Agar

Constituents:

1. Agar.
2. Ethylcinnamic ether.

Preparation:

(1) Liquify 10.0 cc. tubes of nutrient agar by heating on the salt water bath.

(2) Add one small drop of ethyl cinnamic ether to each tube by means of a fine pipette. This is equivalent to 0.025 g.

Sterilization: Not specified.

Use: Differentiation of dysentery bacilli. Author reported that the Hiss type of dysentery failed to develop in the presence of ethyl cinnamic ether while the Flexner showed good development.

Reference: Jacobson (1919 p. 726).

1816. Hurler's Caffeine Agar

Constituents:

1. Nutrient agar	1000.0 cc.
2. Caffeine (0.3%)	3.0 g.

Preparation:

(1) Prepare nutrient agar.

(2) Adjust the reaction of (1) to slightly alkaline.

(3) Add 0.3% caffeine to (2). The caffeine is dissolved in 5.0 cc. of distilled water by heating over the water bath before adding to the agar.

Sterilization: Method not given.

Use: Cultivation of typhoid and intermediate group.

Reference: Hurler (1912 p. 357).

1817. Finger, Ghon and Schlagenhaufer's Urea Agar

Constituents:

1. Nutrient agar (2.0% agar, 1.0% peptone)	1000.0 cc.
2. Urea (2.0%)	20.0 g.

Preparation:

(1) Prepare nutrient agar containing 2.0% agar with 1.0% peptone.

(2) Add 2.0% urea to (1).

Sterilization: Not specified.

Use: Cultivation of gonococci. Author reported that growth was not as luxuriant as in serum agar. Also used by other investigators to study urea decomposition.

Variants:

(a) The author used 3.0 or 5.0% urea instead of 2.0%.

(b) Löhnis studied urea decomposition by adding 1.0 cc. of a 15.0% aqueous solution of urea to each tube of nutrient agar. The medium was then heated in the steam sterilizer.

(c) Cunningham added 2.0, 5.0 or 10.0% urea to nutrient agar, and sterilized by heating intermittently in the steamer. The medium was used to study urea decomposition.

Reference: Finger, Ghon and Schlagenhaufer (1894 p. 14), Löhnis (1913 p. 95), Cunningham (1924 p. 143).

1818. Russell's Double Sugar Agar

Constituents:

1. Nutrient agar (2.0 or 3.0%)	1000.0 cc.
2. Litmus solution (5.0% aqueous solution)	30.0 to 50.0 cc.
3. Lactose	10.0 g.
4. Glucose	1.0 g.

Preparation:

(1) Prepare 2.0 or 3.0% nutrient agar with a reaction of about 0.8% acid to phenolphthalein.

(2) Add enough of a 5.0% aqueous solution of litmus to (1) to give a distinct purple violet, the amount required depending on the original color of the agar.

(3) Adjust the reaction by adding sodium hydrate until the mixture is neutral to litmus.
(4) Add 1.0% lactose and 0.1% glucose (dissolved in a small amount of hot water) to (3).
(5) Slant the tubes following sterilization and store in small quantities in a dark place.

Sterilization: Sterilize in the Arnold. Pack the tubes loosely in the sterilizer basket to allow good circulation of the steam. Under these conditions 10 minutes on the first day and 15 minutes the second are sufficient.

Use: Isolation of typhoid, paratyphoid and dysentery from feces and urine.

Variants:

(a) Kligler gave the following variant:
  (1) Prepare the nutrient agar from meat infusion (or beef extract). (Preferably clear sugar free beef infusion.) Exact composition not given.
  (2) Adjust to pH = 7.4 or neutral to Andrade's indicator.
  (3) Add 1.0% by volume of Andrade's indicator.
  (4) Tube in 5.0 cc. lots and sterilize.
  (5) Prepare a solution containing 20.0% lactose and 20.0% glucose and sterilize.
  (6) Add 0.25 cc. of sterile (5) to each tube of melted sterile (4), cooled to 60°C.
  (7) Prepare and sterilize a 0,25% basic lead acetate solution and add 1.0 cc. to each tube of (4) cooled to 60°C.
  (8) Slant so as to have a butt of at least ½ to ⅝ inches and a slant of about 1½ inches.

Kligler gave the following special reactions: *B. coli* reddened whole tube and gas was produced. *Bacillus typhosus* reddened butt, colorless slant and produced browning particularly near the surface of the stab. The dysentery bacillus reddened the butt but did not produce browning. Paratyphosus bacilli produced gas while *Bacillus typhosus* and *Bacillus dysenteriae* did not. *Bacillus paratyphosus B.* and allied bacilli, *Bacilli enteritidis* and *Bacillus murium* produced browning while A type did not.

(b) Giltner prepared a similar medium as follows:
  (1) Prepare a 1.5% nutrient agar.
  (2) Add 1.0% glucose and 1.0% lactose to (1).
  (3) Make up a 0.5% solution of basic lead acetate.
  (4) Sterilize (3). (Method not given.)
  (5) Tube.
  (6) Add the necessary amount of (4) to each tube of agar to give 0.05% basic lead acetate.
  (7) Sterilize (method not given).

(c) Pitfield substituted Andrade's indicator for litmus as an indicator.

References: Russell (1911 p. 226), Roddy (1917 p. 43), Kligler (1918 p. 321), Tanner (1919 p. 52), Ball (1919 p. 84), Giltner (1921 pp. 385, 386), Harvey (1921–22 p. 109), Pitfield (1922 p. 120), Stitt (1923 pp. 50, 51).

1819. Thoinot and Masselin's Glucose Glycerol Agar

Constituents:

1. Agar	1000.0 cc.
2. Glucose (5.0 to 10.0%)	50.0 to 100.0 g.
3. Glycerol (4.0 to 6.0%)	40.0 to 60.0 g.

Preparation:

(1) Add 5.0 to 10.0% glucose and 4.0 to 6.0% glycerol to agar.

Sterilization: Not specified.

Use: General culture medium.

Variants:

(a) Besson dissolved 20.0 g. agar in a liter of bouillon, and added 2.0 to 4.0% glucose and 5.0% glycerol. The medium was sterilized at 115°C. for 20 minutes.

(b) Dopter and Sacquépée solidified bouillon with agar, and added 5.0% glycerol and 2.0% glucose.

References: Thoinot and Masselin (1902 p. 35), Besson (1920 p. 43), Dopter and Sacquépée (1921 p. 128).

1820. Thoinot and Masselin's Sucrose Glycerol Agar

Same as medium 1819 but substituting 5.0 to 10.0 g. sucrose for glucose.

1821. Kligler and Defandorfer's Double Sugar Agar

Prepared in the same manner as Russell's Double Sugar Medium (see 1818) using the same constituents but substituting 0.5% mannitol for 1.0% lactose. The authors reported they used the medium for the differentiation of B. typhosus from dysentery bacilli.

Reference: Kligler and Defandorfer (1918 p. 439).

1822. Kendall and Ryan's Sucrose Mannitol Agar

Constituents:

1. Nutrient agar 2.5%	1000.0 cc.
2. Mannitol	1.0 g.
3. Sucrose	10.0 g.
4. Andrade indicator	10.0 cc.

Preparation:

(1) Prepare nutrient 2.5% agar.
(2) Adjust to a reaction so that when Andrade indicator is added that the medium be a faint pink while hot.
(3) Add 2 and 3 to (2).
(4) Tube.
(5) Slant so that slanted surface begins 1.0 cm. from the bottom. Medium is colorless when cold.

Sterilization: Not specified.

Use: Detection of intestinal and other bacteria.

Reference: Kendall and Ryan (1919 p. 403).

1823. Hulton-Frankel and MacDonald's Inositol-Dextrin Agar

Constituents:

1. Nutrient agar (3.0%)	1000.0 cc.
2. Inositol (1.0%)	10.0 g.
3. Dextrin (1.0%)	10.0 g.
4. Litmus	

Preparation:

(1) Add 1.0% inositol and 1.0% dextrin to 3.0% nutrient agar.
(2) Add litmus as an indicator.

Sterilization: Not specified.

Use: Differentiation of typhoid group. The authors reported that typhoid fermented dextrin with acid formation in the butt of the tube, decolorizing entirely in 24 hours with a violet slant. Paratyphoid A did not ferment either dextrin or inositol. Paratyphoid B fermented inositol with the formation of gas.

Reference: Hulton-Frankel and MacDonald (1917 p. 31).

1824. Kitasato's Glucose Formate Agar (Tanner)

Constituents:

1. Nutrient agar	1000.0 cc.
2. Glucose	20.0 g.
3. Sodium formate	4.0 g.

Preparation:

(1) Prepare nutrient agar.
(2) Dissolve 2 and 3 in 1.
(3) Adjustment of reaction not given.
(4) Tube.

Sterilization: Method not given.

Use: General culture medium.

Reference: Tanner (1919 p. 49).

1825. Söhngen and Fol's Glucose Butyrate Agar

Constituents:

1. Water agar	50.0 cc.
2. Nutrient agar	50.0 cc.
3. Calcium butyrate	1.0 g.
4. Glucose	0.25 g.

Preparation:

(1) Prepare tap water agar and nutrient agar.
(2) Mix 50.0 cc. of tap water and 50.0 cc. of nutrient agar.
(3) Dissolve 3 and 4 in (2).

Sterilization: Not specified.

Use: Cultivation of actinomyces, *Actinomyces elastica* and *Actinomyces fuscus*

Reference: Söhngen and Fol (1914 p. 95).

1826. MacDonald's Glucose Lactic Acid Agar

Constituents:

1. Nutrient agar	1000.0 cc.
2. Glucose	10.0 g.
3. Lactic acid	1.0 to 10.0 g.

Preparation:

(1) Prepare nutrient agar using Armour's peptone.
(2) Titrate carefully so that after one hour sterilization in the autoclave at 15 pounds pressure, the reaction is neutral to phenolphthalein.
(3) Tube in 10.0 cc. lots.
(4) Cool sterile (3) to 50°C. and add lactic acid in varying amounts to

each tube undiluted so that 0.1 cc. equals 1.0% of acid.

(5) Add glucose.

Sterilization: Sterilize in autoclave at 15 pounds pressure one hour.

Use: To show effect of lactic acid on growth of organisms. Author reported that lactic acid had a varying degree of inhibitory action on bacteria and fungi. Generally 1.0% lactic acid inhibited growth.

Reference: MacDonald (1917 p. 322).

1827. Müller's Lactose Tartrate Agar

Constituents:

1. Bouillon	1000.0 cc.
2. Lactose	25.0 g.
3. Agar	30.0 g.
4. Potassium ferric tartrate	
5. Potassium ferrocyanide	
6. Fuchsin	

Preparation:

(1) Dissolve 30.0 g. of agar and 25.0 g. lactose in 1 liter of bouillon.

(2) Neutralize and then add 10.0 to 12.0 cc. of normal NaOH to (1).

(3) Prepare a 10.0% solution of potassium ferrocyanide.

(4) Prepare a 10.0% solution of potassium ferric tartrate.

(5) Add 50.0 cc. of sterile (4) to 1000.0 cc. of sterile (2). Mix well.

(6) Add 50.0 cc. of sterile (3) to (5). Mix well.

(7) Color the agar by the addition of 10.0 cc. fuchsin solution.

(8) Distribute as desired in sterile containers.

Sterilization: Sterilize (2) in the usual manner (method not given). The lactose may be sterilized at 100°C. in water and then added to the sterile agar if desired. Sterilize (3) by heating at 100° for 30 minutes. Sterilize (4) by heating at 100°C. for 30 minutes.

Use: Differentiation of colon typhoid bacilli. The author gave the following reactions:

With fuchsin, typhoid blue; coli red.

With safranin, typhoid blue; coli orange.

With Bismark brown, typhoid brown, coli bright green.

With G. orange, typhoid brown, coli bright green.

Variants: The author used one of the following instead of 10.0 cc. of fuchsin:

Safranin	10.0 cc.
Bismark brown	50.0 cc.
G. orange	60.0 cc.
Neutral red	60.0 cc.

Reference: Müller (1922 p. 1251).

1828. Kligler's Lead Acetate Glucose Agar

Constituents:

1. Nutrient agar (0.5%)	1000.0 cc.
2. Glucose (0.2%)	2.0 g.
3. Lead acetate (0.05 to 0.1%)	0.5 to 1.0 g.

Preparation:

(1) Add 0.05% to 0.1% lead acetate and 0.2% glucose to 0.5% nutrient agar.

Sterilization: Method not given.

Use: Differentiation of typhoid and paratyphoid bacilli. The author reported that *B. typhi* produced browning without gas. *B. paratyphi B.* produced browning with gas, *B. paratyphi A.* produced no browning and gas and *B. dysenteriae* produced no browning and no gas.

Reference: Kligler (1917 p. 805).

1829. MacConkey's Lactose Bile Salt Agar (Heinemann)

Constituents:

1. Nutrient agar	100.0 cc.
2. Sodium taurocholate (0.5%)	0.5 g.
3. Peptone (2.0%)	2.0 g.
4. Lactose (2.0%)	2.0 g.

Preparation:

(1) Prepare nutrient agar.

(2) Add 2 and 3 to (1).

(3) Boil.

(4) Clarify.

(5) Filter.

(6) Add 2.0% lactose.

(7) Tube.

Sterilization: Method not given.

Use: General culture medium.

Reference: Heinemann (1905 p. 128).

1830. Fleming's Oleic Acid Glycerol Agar

Constituents:

1. Nutrient agar	100.0 cc.
2. Glycerol (2.0%)	2.0 g.
3. Oleic acid (0.1%)	0.1 g.

Preparation:

(1) Neutralize nutrient agar with HCl.

(2) Add 2.0% glycerol and 0.1% oleic acid to (1).
(3) Tube.

Sterilization: Sterilize in the autoclave in the usual way. (Details not given.)

Use: Cultivation of the bacillus of acne vulgaris (*Bacillus acne*).

Reference: Fleming (1909 p. 1036).

1831. Rosenow and Towne's Ascitic Fluid Kidney Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte's)	
3. Meat extract (Liebig's)	
4. Glucose	10.0 g.
5. Ascitic fluid (10.0%)	100.0 cc.
6. Rabbit kidney, sterile	
7. Agar	25.0 g.

Preparation:
(1) Prepare an extract broth from water, Liebig's Beef Extract and Witte's peptone (amounts not given).
(2) Titrate and adjust the reaction of (1) to 0.6–0.8 acid to phenolphthalein.
(3) Add and dissolve 1.0% glucose and 2.5% agar in (2).
(4) Add 10.0% sterile ascitic fluid to sterile (3).
(5) Distribute in tall test tubes (column of medium measuring 0.8 cc. in diameter and 13.0 cc. in height.
(6) Add a piece of fresh sterile rabbit kidney.
(7) Add a layer of sterile mineral oil.

Sterilization: Method of sterilization of (3) not given.

Use: Cultivation of pleomorphic streptococci causing poliomyelitis. The author reported that initial growth could best be obtained without the addition of the kidney.

Variants: The authors prepared the medium as indicated in step (1) thru (4), omitting the agar. One part of this ascitic fluid broth was mixed with 1.5% agar, tubed and a piece of kidney added to each tube. A layer of sterile mineral oil was added to each tube.

Reference: Rosenow and Towne (1917 p. 177).

1832. Rosenow's Glucose Brain Agar (Haden)

Constituents:

1. Water	1000.0 cc.
2. Bacto nutrient broth	8.0 g.
3. NaCl	8.0 g.
4. Glucose (c.p.)	2.0 g.
5. Andrade indicator	10.0 cc.
6. Brain, calf	
7. Marble	
8. Agar	7.0 g.

Preparation:
(1) Dissolve 2, 3 and 8 in 1.
(2) Cool and add 4 and 5.
(3) Tube in 6 by 0.75 inch test tubes so that the depth is at least 3.4 to 4.0 inch.
(4) Add 3 pieces of crushed calf brain about 1 cmm. square and two or three pieces of crushed marble to each tube.

Sterilization: Autoclave for 20 minutes at 15 pounds pressure.

Use: Isolation of bacteria from infected teeth.

Reference: Haden (1923 p. 831).

1833. Goldberger's Glucose Alkaline Egg Agar (Abbott)

Constituents:

1. Distilled water	1000.0 cc.
2. Egg	1.0
3. Meat extract (Liebig's)	3.0 g.
4. Peptone (Witte's)	10.0 g.
5. NaCl (c.p.)	5.0 g.
6. Glucose	1.0 g.
7. Agar	30.0 g.

Preparation:
(1) Mix a whole egg with an equal volume of distilled water.
(2) Mix one volume of (1) with an equal volume of 6.5% solution of anhydrous Na_2CO_3.
(3) Steam for 30 to 60 minutes.
(4) Mix 3, 4, 5, 6 and 7 in 1000.0 cc. of water.
(5) Steam for 3 hours to insure complete solution.
(6) Decant or filter thru cotton.
(7) Distribute in 100.0 cc. quantities in flasks.
(8) Mix one volume of (3) with five volumes of sterile (7). The agar

should be completely liquified by steam.

(9) When thoroly mixed, pour into Petri dishes.

(10) Place in the incubator with covers partially removed.

Sterilization: Sterilize (7) by steaming for 90 minutes.

Use: Diagnosis of cholera. Abbott reported that cholera vibrio and cholera-like organisms grew luxuriantly while other organisms were restrained.

Variants: Krumwiede (Park, Williams and Krumwiede) prepared a similar medium as follows:

(1) Mix equal parts egg and water.

(2) Mix equal parts (1) and a 12.0% Na_2CO_3 (crystalline) solution.

(3) Steam in the Arnold sterilizer for 20 minutes.

(4) Prepare a 3.0% beef extract peptone (Fairchild's) agar. Do not adjust the reaction. The size of the colonies is increased if 0.2% glucose be added.

(5) Mix 20 parts (3) to 80 parts (4). Sterilization not specified.

(6) Pour in plates.

(7) Dry for 20 minutes.

Reference: Abbott (1921 p. 571), Park, Williams and Krumwiede (1924 p. 130).

1834. Goldberg's Meat Infusion Extract Agar (Stitt)

Constituents:

1. Extract agar (3.0%)	1500.0 cc.
2. Water	500.0 cc.
3. Beef	500.0 g.

Preparation:

(1) Treat 500.0 g. lean beef with 500.0 g. water. Temperature not specified.

(2) After 3 hours strain.

(3) Adjust the reaction to neutral by the addition of 5.3% anhydrous Na_2CO_3 solution.

(4) Add 2.5 cc. of 5.3% anhydrous Na_2CO_3 solution for each 100.0 cc. of infusion.

(5) Filter sterile (4).

(6) Prepare a 3.0% meat extract agar.

(7) Mix one volume of (5) with 3 volumes of hot melted sterile (6).

(8) Pour plates.

(9) Cover the plates with a piece of filter paper and place in the incubator for 30 minutes until they are dry.

Sterilization: Sterilize (4) in the Arnold for 30 minutes. Sterilization of extract agar not specified.

Use: Cultivation of cholera organisms. Stitt reported that cholera colonies were clear, round and showed a brownish center. They did not show the striking bluish opalescence as on ordinary plates.

Reference: Stitt (1923 p. 50).

1835. Dimitroff's Egg Agar

Constituents:

1. Extract agar	750.0 cc.
2. Extract broth	250.0 cc.
3. Egg white	

Preparation:

(1) Place a small cube of hard boiled egg white in 10.0 cc. of beef extract broth.

(2) Following sterilization of (1), mix 25.0 cc. of (1) in 75.0 cc. of beef extract agar.

Sterilization: Method of sterilization not given.

Use: Cultivation of *Spirillum virginianum*, The author reported that the spirilla produced small dew-drop, convex, glistening, slightly opaque colonies.

Reference: Dimitroff (1926 p. 22).

1836. Dunschmann's Bile Salt Gelatin Agar

Constituents:

1. Meat extract	1000.0 cc.
2. Agar (3.0 to 4.0%)	30.0 to 40.0 g.
3. Gelatin (0.5%)	5.0 g.
4. Sodium taurocholate (1.5 to 2.5%)	15.0 to 20.0 g.
5. Lactose (4.0%)	40.0 g.
6. Peptone (5.0%)	50.0 g.
7. Litmus	

Preparation:

(1) Prepare a meat extract with Liebig's beef extract (or by extracting 500.0 g. of veal with 1 liter of water).

(2) Dissolve 2, 3, 4, 5 and 6 in (1).

(3) Distribute in flasks or Petri dishes and add 10.0% sensitive litmus (strength of solution not specified).

Sterilization: Not specified.

Use: Enrichment medium for typhoid bacilli. Author reported that mineral salts may be used as a basis for this medium instead of extract or infusion broth.

Reference: Dunschmann (1909 p. 64).

1837. Paneth's Glucose Ascitic Fluid Agar

Constituents:

1. Distilled water	3000.0 cc.
2. Liebig's meat extract	12.0 g.
3. NaCl	15.0 g.
4. Peptone (Witte's)	30.0 g.
5. N/1 H_2SO_4	17.5 cc.
6. Agar	85.0 g.
7. Glucose	50.0 g.
8. Ascitic fluid	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Add 6 to (1).
(3) Allow to stand for several hours.
(4) Boil in the steamer for two hours.
(5) Filter thru gauze.
(6) Mix the whites of eight eggs with 150.0 cc. distilled water.
(7) Cool the liquid agar to 50°, add (6) and mix well.
(8) Pour into flasks and heat at 100° in the steamer for two hours.
(9) Filter thru wet cotton. Gauze is placed on top of the cotton.
(10) Adjust the reaction from 1.8 to 2.2.
(11) Add 2.0% glucose (about 50.0 g.).
(12) Heat for 20 minutes in a steamer.
(13) Distribute into large test tubes in about 15.0 to 20.0 cc. lots.
(14) Distribute sterile ascitic fluid into large sterile test tubes.
(15) Liquify the agar and cool to 40 to 42°C.
(16) Draw, under aseptic conditions, about 2.0 cc. of infected blood directly into the liquefied agar and add one tube of ascitic fluid (amount not specified) to the agar. Mix. (In the work of Plotz, Olitsky and Baehr, 2.0 cc. of ascitic fluid is added.)
(17) After cooling cover with plain agar or paraffin.

Sterilization: Sterilize (13) for 20 minutes in the autoclave at 10.0 kilograms pressure or in the steamer on two or better four successive days for 20 minutes each day.

Use: Cultivation of *Bacterium typhi exanthematis.*

Reference: Paneth (1916 p. 647).

1838. Plotz, Olitsky and Baehr's Ascitic Fluid Agar

Constituents:

1. Glucose agar (2.0%)	1000.0 cc.
2. Ascitic fluid	200.0 cc.
3. Plain agar	

Preparation:

(1) Prepare nutrient agar using Liebig's meat extract and containing 2.0% glucose and 2.0% agar.
(2) Distribute in 20.0 cc. lots in tubes 15.0 cm. x 2 cm.
(3) Cool the sterile melted agar tubes to 40°C. and add to each tube 4.0 cc. ascitic fluid and 2.0 cc. of infected blood.
(4) Mix by pouring back and forth into sterile test tube, avoiding air bubbles.
(5) When the agar has solidified add sterile plain agar to a depth of 2.0 cm. to each tube.
(6) Incubate at 37.5°C.

Sterilization: Method not given.

Use: Cultivation of *B. typhi exanthematici* (supposed cause of typhus fever), from blood.

Variants: Baehr and Plotz gave the following method of preparation:

(1) Mix 12.0 g. Liebig's meat extract, 15.0 g. NaCl, 30.0 g. Witte's peptone, 17.5 g. N/1 H_2SO_4, and 85.0 g. agar shreds in 3000.0 cc. water.
(2) Place in an Arnold steam sterilizer for two hours.
(3) Filter rapidly thru several layers of gauze to remove water.
(4) Mix the whites of eight eggs with 150.0 cc. distilled water.
(5) Cool (3).
(6) Mix (5) and (4) and shake thoroly.
(7) Place in Arnold sterilizer (100°C.) for two hours.
(8) Decant clear agar and filter thru moistened absorbent cotton.
(9) Adjust to acidity of 0.9 to 1.1 to phenolphthalein.
(10) Add 2.0% glucose to (9).
(11) Distribute into 2 x 20 cm. test tubes in about 20.0 cc. lots (half full).
(12) Sterilize (11) at 10.0 kg. pressure for 20 minutes or in an Arnold on 3 or 4 successive days for 20 minutes.

(13) Remove blood aseptically with a syringe from the median basilic or median cephalic veins of patient (15.0 cc.).

(14) Divide the 15.0 cc. blood among eight sterile melted tubes of (11), 2.0 cc. per tube.

(15) Add ascitic fluid to each tube of (13), ⅓ to ½ volume (i.e., 6–10.0 cc.) ascitic fluid must be clear, free from bile or blood pigment and have a specific gravity of more than 1.015. Must not be filtered, sterilized or contain preservatives.

(16) Mix the content of each tube thoroly by pouring once or twice into another sterile tube. Avoid air bubbles.

(17) After thoro solidification cover each one of the (15) with 2 or 3 cm. of sterile melted agar (11).

(18) To prevent drying, paraffin the cotton stopper of each tube.

References: Plotz, Olitsky and Baehr (1915 p. 6), Baehr and Plotz (1917 p. 203).

1839. Reed and Orr's Blood Agar

Constituents:

1. Water	1000.0	cc.
2. Peptone	5.0	g.
3. Agar	15.0	g.
4. Beef extract	3.0	g.
5. Rabbits blood (whole)	10.0	cc.
6. Phosphates to	0.05M	

Preparation:

(1) Prepare beef extract peptone agar according to Committee A. P. H. A. (1916–1917). (See medium 1695.)

(2) Adjust with NaOH to pH = 7.4.

(3) Add phosphate mixture of pH = 7.4 to make finished medium 0.05M phosphate.

(4) Add whole rabbits blood to sterile (3) cooled to 80°C. to make finished medium 1.0% blood.

(5) Maintain at 80°C. for 10 minutes.

(6) Tube or plate.

Sterilization: Sterilize in the autoclave.

Use: To maintain cultures of *Hemophilus influenzae*. Author reported that pH range of 6.8 to 7.4 gave very nearly maximum growth. Marked morphological changes did not appear except near the limiting pH values.

Reference: Reed and Orr (1923 p. 104).

1840. Becker's Defibrinated Blood Agar

Constituents:

1. Extract agar	1000.0 cc.
2. Blood, defibrinated	175.0 cc.

Preparation:

(1) Prepare extract agar according to Standard Methods, see medium 1695, and distribute in 40.0 cc. lots in 100.0 cc. flasks.

(2) Melt agar and cool to 45–50°C.

(3) Add 1.0 cc. of defibrinated human blood per 6.0 cc. agar.

(4) Mix thoroly.

Sterilization: Method not specified.

Use: To isolate streptococci, pneumococci and gonococci.

Reference: Becker (1916 p. 759), Tanner (1919 p. 69).

1841. Esch's Alkaline Hemoglobin Ragit Agar

Constituents:

1. Distilled water	7.5 cc.
2. NaOH (normal	7.5 cc.
3. Hemoglobin (Merck)	2.5 g.
4. Ragit agar	85.0 cc.

Preparation:

(1) Grind 2.5 g. Merck's hemoglobin to a powder in a mortar.

(2) Place in a balloon flask and add 7.5 cc. of N/1 caustic soda solution and 7.5 cc. of distilled water.

(3) Heat by means of a gas burner and stir strongly to obtain complete solution of the hemoglobin which requires about 10 minutes.

(4) Heat (3) for one hour in a Koch steamer, or for 15 minutes in an autoclave at one atmosphere pressure.

(5) Prepare "Ragit agar."

(6) Melt sterile (5) and add (4) to 85.0 cc. of the agar.

(7) Mix well and distribute into sterile petri dishes.

Sterilization: Step (4) above gives sterilization of alkaline hemoglobin. Method of sterilization of Ragit agar not given.

Use: Enrichment medium for cholera vibrio.

Reference: Galli-Valerio (1912 p. 550).

1842. Padlewsky's Malachite Green Bile Agar

Constituents:

1. Meat extract agar (3.0%)	1000.0 cc.
2. Peptone	20.0 g.
3. Bile	35.0 cc.
4. Lactose (c.p.)	10.0 g.
5. Malachite green (1.0% aq. solution) (Höchst)	5.0 cc.
6. Sodium sulphite (10.0% aq. pur. solution)	7.5 to 10.0 cc.

Preparation:

(1) Prepare a 3.0% meat extract agar. (Meat infusion may be used instead of meat extract).

(2) Treat the ox bile with steam in a Koch's apparatus. (Time not specified).

(3) Filter the bile thru cotton.

(4) Add 2.0% peptone, 3.0% (3) and 1.0% c.p. lactose to (1).

(5) Adjust the reaction to slightly alkaline to litmus.

(6) It is not necessary to filter the agar after the addition of the filtered bile.

(7) Distribute into flasks in 100 to 200 cc. lots.

(8) When ready for use melt the sterile agar, cool to 60 to 65°C.

(9) Add to each 100.0 cc. of (8) 0.5 cc. of a 1.0% watery solution of Höchst chemically pure crystalline malachite green, 0.5 cc. bile and 0.75 to 1.0 cc. of a 10.0% watery solution of sodium sulphite (pur.) by means of a pipette.

(10) Mix thoroly.

(11) Pour into sterile Petri dishes and allow the agar to solidify with the cover of the dish removed.

(12) Dry in the incubator.

Sterilization: Sterilize (7) by the fractional method.

Use: Detection of typhoid group bacilli. The author reported that typhoid colonies were colorless. The medium was transparent and light green. A great many of the intestinal forms were entirely inhibited.

Reference: Padlewsky (1908 p. 543).

1843. Fildes' Body Fluid Agar

Constituents:

1. Extract agar.
2. Ascitic fluid or other body fluid.

Preparation:

(1) Collect ascitic fluid in a large glass bottle and allow to stand in the ice chest for a day or two.

(2) Decant or filter thru glass wool.

(3) Adjust the reaction to slightly alkaline or neutral to litmus by the addition of alkali or acid.

(4) Distribute in measured glass stoppered bottles in 200.0 cc. lots. (The fit of the glass stopper must be perfect.)

(5) Add chloroform to each bottle until 0.5% chloroform has been added.

(6) Add a drop of sterile oil to the stopper and fasten a dust cover tightly over the stopper.

(7) Place the bottles in the water bath for one hour at 45°C. Shake occasionally.

(8) When bottles have cooled, remove a sample under aseptic conditions and mix with agar. Incubate the mixture at 37°C. for 5 days to test sterility.

(9) Prepare ordinary lemco (or meat infusion) agar containing 2.5 to 3.0% agar.

(10) Measure (9) into 200.0 cc. flasks in 150.0 cc. lots.

(11) When ready for use melt in the steamer and treat in one of the following two ways:

  (a) Pour into a sterile distributer maintained at 50°C. by means of a water bath and add 50.0 cc. of ascitic fluid (8) for each flask of agar. Mix and tube thru a "hooded" pipette.

  (b) Pour 50.0 cc. of ascitic fluid (8) directly into a flask of melted agar. Mix well, and distribute into tubes or Petri dishes.

Mix the ascitic fluid and agar at as low a temperature as possible. This aids the production of a transparent medium.

Sterilization: Sterilize (10) in the autoclave.

Use: Cultivation of meningococci and other pathogenic cocci.

Variants:

(a) Fildes substituted serum for ascitic fluid. The serum was prepared as follows:

(1) Collect horse or ox blood in tall sterile jars from the slaughter house.

(2) Allow to stand until all the corpuscles have deposited. It may require 14 days for this to take place. After 5 days examine the jars and pipette or siphon off the clear serum. Do not disturb the sediment.

(3) Distribute the clear serum in 200.0 cc. lots in perfectly fitting glass stoppered flasks.

(4) Add chloroform to each bottle until 0.5% chloroform has been added.

(5) Add a drop of sterile oil to the stopper and fasten a dust cover tightly over the stopper.

(6) Place the bottles in the water bath for one hour at 45°C. Shake occasionally.

(7) When the bottles have cooled, remove a sample under aseptic conditions and mix with agar. Incubate the mixture at 37°C. for five days to test sterility.

(b) Fildes also prepared the medium as follows:

(1) Collect ox or horse blood from a slaughter house.

(2) Defibrinate the blood by stirring with a sterilized large wooden stick wrapped in gauze.

(3) Lake the blood by the addition of an equal volume of distilled water.

(4) Distribute in 200.0 cc. lots into perfectly fitting glass stoppered bottles. (The author mentions that the medium should contain no suspended material, or it will not sterilize with the method employed.)

(5) Add chloroform to each bottle until 0.5% chloroform has been added.

(6) Add a drop of sterile oil to the stopper and fasten a dust cover tightly over the stopper.

(7) Place in the air incubator at 37°C. for 24 hours, shaking constantly.

(8) After this time, remove a sample under aseptic conditions and test its sterility by mixing it with agar and incubating. (Time not specified.) If sterile do not heat longer for heating tends to darken the medium.

(9) Prepare ordinary lemco (or meat infusion) agar containing 2.5 to 3.0% agar.

(10) Measure (9) into 200.0 cc. flasks in 150.0 cc. lots.

(11) Autoclave (time and pressure not given).

(12) Add 25.0% of sterile laked blood (8) to (11). (Method of distribution not specified.)

Reference: Fildes (1917 p. 492).

1844. Haner and Frost's Milk Body Fluid Agar

Constituents:

1. Nutrient agar 1.0% (dehydratedDifco)	1000.0 cc.
2. Milk, sterile.......	1000.0 cc.
3. Serum (horse or rabbit 6.0 to 12.0%).	120.0 to 240.0 cc.

Preparation:

(1) Prepare 1.0% agar from Difco dehydrated product.

(2) Add equal part of sterile milk to sterile (1).

(3) Add 6.0% to 12.0% of sterile horse or rabbit serum to (2).

Sterilization: Method of sterilization not given.

Use: Cultivation of pneumococci and streptococci.

Variants: The authors used whole blood instead of serun.

Reference: Haner and Frost (1921 p. 270).

1845. Bacto Conradi-Drigalski Agar (Dehydrated)

Constituents:

1. Distilled water.	
2. Peptone, Bacto	10.0 g.
3. Sodium caseinate, Bacto	10.0 g.
4. Lactose, Bacto	10.0 g.
5. Agar, Bacto	15.0 g.
6. NaCl	5.0 g.
7. Dibromcresolsulphonephthalein	0.03 g.
8. Crystal violet	0.02 g.

Preparation:

(1) Dissolve 50.0 g. of Bacto Conradi-Drigalski Agar (dehydrated) in 1000.0 cc. of distilled water by boiling or autoclaving.

(2) Restore any lost weight.

(3) If sterilized at 15 pounds pressure for 20 minutes pH = 6.8±.

Sterilization: Sterilize at 15 pounds pressure for 20 minutes.

Use: The author reported that acid producing colonies were yellow.

Reference: Digestive Ferments Co. (1925 p. 12).

1846. Haner and Frost's Milk Agar

Constituents:

1. 1.0% nutrient agar (Dehydrated Difco)	1000.0 cc.
2. Milk, sterile	1000.0 cc.

Preparation:

(1) Prepare 1.0% agar from dehydrated Difco product.

(2) Add an equal part of sterile milk to sterile (1).

Sterilization: Method not given.

Use: Cultivation of staphylococci.

Reference: Haner and Frost (1921 p. 270).

1847: Eldredge and Roger's Whey Agar

Constituents:

1. Whey	1000.0 cc.
2. Lactic acid (10.0%)	20.0 cc.
3. Peptone (1.0%)	10.0 g.
4. Beef extract (5.0%)	50.0 g.
5. Agar (1.2%)	12.0 g.

Preparation:

(1) Heat one liter of skim milk to boiling and add 20.0 cc. of a 10.0% lactic acid solution.

(2) Filter.

(3) Add 1.0% peptone, 5.0% beef extract and 1.2% agar to the filtrate.

(4) Heat to boiling point until all the agar is dissolved.

(5) Correct the reaction to 1.2 using phenolphthalein as an indicator.

(6) Cool to 45°C. and add the whites of an egg.

(7) Heat to 100°C. for 45 minutes and filter thru cotton.

(8) Correct the reaction if necessary and tube.

Sterilization: Sterilize three successive days in the Arnold sterilizer.

Use: Cultivation of bacteria found in cheese. Author reported that bulgaricus types grew well on this medium.

Reference: Eldredge and Rogers (1914 p. 8).

1848. Ayres and Mudge's Milk Powder Agar

Constituents:

1. Water	500.0 cc.
2. Skimmed milk powder.	5.0 g.
3. $Na_2HPO_4 + 2H_2O$ (Sorensen's phosphate).	1.0 g.
4. Peptone (Difco)	2.0 or 5.0 g.
5. Liebig's extract	1.0 or 3.0 g.
6. Agar 3.0%	500.0 cc.

Preparation:

(1) Pour 5.0 g. skimmed milk powder into 20.0 cc. cold distilled water. Stir until dissolved.

(2) Dissolve by heat, 1.0 g. Sorensen's phosphate in 5.0 cc. distilled water.

(3) Add (2) to (1), place in 30°C. water bath and heat to 60° in 10 minutes. A flocculent grayish precipitate appears.

(4) Steam until precipitate gets white.

(5) Dilute about ⅓ with distilled water and steam 5 more minutes.

(6) Decant solution and filter, keeping most of the precipitate in beaker until most of the liquid has passed thru the filter. Then wash precipitate with a little distilled water. The filtrate is cloudy.

(7) Make up (6) to 250.0 cc. with distilled water.

(8) Dissolve 5.0 g. peptone and 3.0 g. Liebig's extract in 100.0 cc. distilled

water by steaming in Arnold, or boiling over flame.

(9) Filter until clear and make up to 250.0 cc. with distilled water.
(10) Mix (7) and (9).
(11) Place 30.0 g. agar in 2000.0 cc. distilled water and let stand 24 hours.
(12) Pour off water and add 2000.0 cc. more water.
(13) Allow to stand 24 hours, pour on cotton flannel cloth in funnel and wash once more with one liter distilled water. Press out as much water as possible.
(14) Add enough water to have weight of 1030.0 cc. of agar and water.
(15) Dissolve in Arnold and filter thru cotton flannel until clear.
(16) Add 500.0 cc. of (15) to (10).

Sterilization: Method not given.

Use: To determine number of bacteria in milk. Authors reported that the medium gave high count. Colonies were large and easy to count.

Variants: The authors specified that indicators and glucose or lactose might be added if desired.

Reference: Ayres and Mudge (1920 p. 568).

1849. Conradi-Drigalski's Crystal Violet Litmus Agar (Park, Williams and Krumwiede)

Constituents:

1. Water	1000.0 cc.
2. Agar	20.0 g.
3. NaCl	5.0 g.
4. Peptone	20.0 g.
5. Nutrose	10.0 g.
6. Beef extract (Liebig's)	4.0 g.
7. NaOH (N/1)	50.0 cc.
8. Litmus (Kubel and Thiemann's)	130.0 cc.
9. Crystal violet (1:1000)	10.0 cc.
10. Lactose	15.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1 in an autoclave.
(2) Cool and clarify with eggs.
(3) Adjust the reaction moderately but distinctly alkaline to litmus.
(4) To each liter of (3) add 130.0 cc. of Kubel and Thiemann's litmus solution, 10.0 cc. of a 1 to 1000 solution of crystal violet, and 15.0 g. of lactose.
(5) Heat in an Arnold sterilizer 10 minutes to obtain thoro mixing.
(6) Distribute in tubes or bottles.

Sterilization: Sterilize in the Arnold sterilizer.

Use: Differentiation of colon-typhoid group. Author reported that acid production indicated by black colonies, or colonies having black centers. Omit the saccharose when used for dysentery isolation. *B. dysenteriae* (Shiga) may fail to grow on this medium. Omit the crystal violet if to be used for dysentery.

Reference: Park, Williams and Krumwiede (1924 p. 128).

1850. Tausz and Peter's Ragit Nutrose Agar

Constituents:

1. Water	1000.0 cc.
2. Ragit nutrose agar	52.0 g.
3. Congo red tablets (Merck).	18.0 g.

Preparation:

(1) Boil 52.0 g. ragit nutrose agar in one liter of water for one hour in the steamer. Shake occasionally.
(2) Filter.
(3) Distribute in 100.0 cc. lots.
(4) To each 100.0 cc. of (3) add 1.8 g. of Congo red tablets (Merck). The tablets contain lactose and congo red.
(5) Boil for 15 or 20 minutes.
(6) Pour into Petri dishes.

Sterilization: Not specified.

Use: Cultivation of *Bacterium aliphaticum*, *Bacterium aliphaticum liquefaciens*, paraffin bacteria. Authors reported that ragit nutrose agar is a commercial powder. Congo red tablets contain lactose and congo red.

Reference: Tausz and Peter (1919 p. 508).

1851. Hunter's Trypsinized Casein Extract Agar

Constituents:

1. Water	1000.0 cc.
2. Casein	15.0 g.
3. Peptone	15.0 g.
4. Beef extract	3.0 g.
5. Agar	15.0 g.
6. Lactose	15.0 g.
7. Sodium oleate (2.0% soln.)	50.0 cc.

Preparation:

(1) Dissolve 2, 3, and 4 in 1.
(2) Add 1.5 g. trypsin and make slightly alkaline.
(3) Add chloroform.
(4) Incubate at 37°C. for 48 hours and then boil several minutes to remove the chloroform.
(5) Add 15.0 g. agar.
(6) Adjust the reaction to pH 7.0 and add 15.0 g. lactose and 50.0 cc. of a 2.0% sodium oleate solution. (In another part of the article the author states that best growth was obtained with about 1.0% sodium oleate. The percentage used above is only 0.1%.
(7) Filter.
(8) Tube.

Sterilization: Sterilize in the autoclave.

Use: Cultivation of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus*.

Reference: Hunter (1924 p. 3).

1852. Zoller's Citrate Milk Agar

Constituents:

1. Distilled water	5000.0 cc.
2. Bacto Nutrient Agar (1.0%)	120.0 g.
3. Sodium citrate	2.0 g.
4. Milk (skimmed) powder	25.0 g.
5. $Na_2HPO_4 \cdot 2H_2O$	6.0 g.
6. Lactose	5.0 g.

Preparation:

(1) Stir 120.0 g. of Bacto nutrient agar (1.0%) into 4 liters of distilled water.
(2) Add 2.0 g. sodium citrate and set in streaming steam in the autoclave for about 5 minutes.
(3) Stir 25.0 g. of any good grade of milk powder in 100.0 cc. distilled water.
(4) Stir 6.0 g. $Na_2HPO_4 \cdot 2H_2O$ in 30.0 cc. of distilled water and heat to boiling.
(5) Add (4) to (3) and steam for 5 minutes at the same time the agar is being steamed.
(6) Remove the agar from the autoclave and stir.
(7) Add 50.0 cc. distilled water to (5) following steaming.
(8) Replace (6) and (7) in the autoclave and heat for 5 minutes at 5 pounds pressure.
(9) Filter the milk phosphate solution thru a small piece of absorbent cotton placed in the neck of a funnel.
(10) Add 5.0 g. lactose to filtered (9) and make up to one liter by the addition of distilled water.
(11) Mix (10) and the agar from (8).
(12) Distribute as desired.

Sterilization: Autoclave at 15 pounds pressure for 15 minutes, or sterilize fractionally by heating at 5 pounds pressure for 10 minutes on each of 2 successive days holding the medium at 30°C. between heating.

Use: Qualitative milk counts.

Reference: Zoller (1923 p. 385).

1853. Smillie's Tissue Infusion Agar

Constituents:

1. 2.0% veal infusion agar	100.0 cc.
2. Ascitic fluid	200.0 cc.
3. Rabbit tissue (kidney)	

Preparation:

(1) Prepare 2.0% veal infusion agar.
(2) Adjust (1) to +0.5 to phenolphthalein
(3) When ready for use, melt sterile (2), boil and cool rapidly to 50°C.
(4) Warm, clear, straw colored, bile free, ascitic fluid having a specific gravity of 1.015 or higher to 45°C.
(5) Place in a vacuum to remove air.
(6) Mix one part melted (3) at 50°C. and two parts (5) at 45°C.
(7) Add 15.0 cc. of (6) to a sterile tube containing sterile rabbit tissue (kidney) (and the poliomyelitis materials).

Sterilization: Method not specified.

Use: Cultivation of globoid bodies of poliomyelitis. Author reported that the organisms did not attack any added carbohydrate. Other investigators used similar media for the cultivation of anaerobes.

Variants:

(a) Olitsky and Gates cultivated anaerobic organisms found in influenza on a medium prepared as follows:
  (1) Prepare a 2.0% beef infusion agar.
  (2) Adjust (1) to pH = 7.4.
  (3) Mix one part (2) with two parts sterile human ascitic fluid in a flask at 40°C.

(4) Pipette (3) into sterile tall test tubes containing pieces of sterile rabbit kidney and the inoculum.
(5) Seal with sterile melted vaseline.

(b) Harvey cultivated anaerobic spirochaetes on the following medium:
(1) Transfer small portions of organs aseptically removed from a rabbit, specially killed for the purpose (2514 variant (a), to each sterile test tube 20 x 2 cm.
(2) Mix one part infusion agar, 1.0% acid to phenolphthalein at 45°C. (see medium 1661 variant (v) for preparation) with one part sterile ascitic fluid heated to 45°C.
(3) Fill, with sterile precautions, about 20.0 cc. of (2) into each test tube (1).
(4) Cover the medium to a depth of 3 cm. with sterile liquid paraffin.
(5) Test sterility by incubation 48 hours.

References: Smillie (1918 p. 324), Olitsky and Gates (1921 p. 715), Harvey (1921–22 p. 97).

1854. van Riemsdyk's Liver Infusion Agar

Constituents:

1. Water	500.0 cc.
2. Beef liver	0.5 lb.
3. Peptone (Witte)	5.0 g.
4. NaCl	2.5 g.
5. Agar	10.0 g.
6. 2.0% glucose agar	500.0 cc.
7. Liver tissue	
8. Glucose	10.0 g.
9. Chalk	

Preparation:
(1) Pass raw beef liver thru a fine meat grinding machine.
(2) Add 500.0 cc. of water to ½ pound of (1).
(3) Boil for 30 minutes with occasional stirring. Allow to settle.
(4) Pour off the liquid and force the liver thru a very fine metallic sieve.
(5) Add the pulverized liver and 3, 4 and 5 to the liquid.
(6) Heat for one hour at 110°C. and make alkaline to phenolphthalein using N/10 NaOH.
(7) Add 10.0 g. of glucose and mix with an equal volume of 2.0% glucose agar.
(8) Add sterile chalk in excess (about 5 spoonfulls) and distribute into sterile tubes.
(9) Add a sterile piece of liver to each tube.

Sterilization: Sterilize at 100°C. for one hour and slant.

Use: Cultivation of *Bact. butyricus* and other anaerobes.

Reference: van Riemsdyk (1922 p. 248).

1855. Williams' Tissue Infusion Agar

Constituents:

1. Water, tap	90.0 cc.
2. Agar (1.0%)	1.0 g.
3. Infusion broth (10.0%)	10.0 cc.
4. Brain	

Preparation:
(1) Mix 90.0 cc. tap water, 10.0% ordinary infusion broth and 1.0% agar.
(2) Dissolve.
(3) Reaction neutral to phenolphthalein.
(4) Cut fresh sterile brain in small pieces and place on the surface of sterile (3).

Sterilization: Sterilize as usual (method not given).

Use: Cultivation of amoeba.

Variants: Park, Williams and Krumwiede substituted liver or kidney for brain.

Reference: Park, Williams and Krumwiede (1924 p. 134).

1856. Olitsky and Gates' Ascitic Fluid Kidney Agar

Constituents:
1. Beef infusion broth.
2. Beef infusion (2.0%) agar.
3. Human ascitic fluid.
4. Rabbit kidney.

Preparation:
(1) Prepare beef infusion broth and 2.0% beef infusion agar.
(2) Adjust both the broth and agar to pH = 7.4.
(3) Place in each 50.0 cc. Florence flask ¼ of a moderate size rabbit kidney. Sections are cut across the entire kidney and placed with the cut surface parallel to the base of the flask.
(4) Inoculate the kidney.
(5) Mix one part (2.0%) infusion agar, (2), with two parts sterile human ascitic fluid of pH = 7.8 to 8.0, in a flask at 40°C.

(6) Add 100. cc. of (5) to each flask.
(7) Solidify the agar by immersing the flask in cold water for 15 minutes.
(8) Mix one part infusion broth (2) and two parts sterile ascitic fluid.
(9) Fill each flask (7) to the neck with the mixture of (8).
(10) Seal with a layer of sterile vaseline 1.0 cm. deep.

Sterilization: Methods of sterilization not specified.

Use: Mass cultivation of anaerobic organisms found in influenzae.

Reference: Olitsky and Gates (1921 p. 716).

1857. Pelouze and Viteri's Brain Veal Infusion Agar

Constituents:

1. Dist. water	500.0 cc.
2. Brain, calf	500.0 g.
3. KH_2PO_4 (0.5%)	2.5 g.
4. Peptone (1.0%)	5.0 g.
5. Veal infusion agar (2.5%)	

Preparation:

(1) Force 500.0 g. of calf brain thru a wide meshed gauze into 500.0 cc. distilled water and place in the ice box for 24 hours.
(2) Filter thru cotton of varying degrees of compactness. The filtrate will not be clear.
(3) Add 0.5% KH_2PO_4 and 1.0% peptone.
(4) Prepare standard 2.5% agar from veal infusion with the addition of 0.5% NaCl and 1.0% peptone.
(5) Mix one part (2) with three parts (4).
(6) Adjust to pH 7.8.
(7) Tube.
(8) Following sterilization and solidifying replace the cotton plug with a sterile rubber cork.

Sterilization: Sterilize in the autoclave.

Use: Cultivation of gonococci.

Variants: Following the mixing of the brain and agar the medium may be placed in the autoclave, brought to 15 pounds pressure quickly and then filtered before tubing and sterilizing. This process gives a clear medium but gives also a scanty growth. The author does not recommend the clear medium.

Reference: Pelouze and Viteri (1926 p. 685).

1858. Avery's Oleate Blood Cell Agar (Harvey)

Constituents:

1. Infusion agar	950.0 cc.
2. Sodium oleate (2.0% neutral solution)	50.0 cc.
3. Erythrocytes, human	10.0 cc.

Preparation:

(1) Prepare infusion agar according to Harvey's method (see variant (v) medium 1661).
(2) Adjust (1) to 0.4% acid to phenolphthalein and heat to 45°C.
(3) Mix 950.0 cc. (2) with 50.0 cc. of a 2.0% neutral sodium oleate solution, and 10.0 cc. of sterile washed human erythrocytes, both heated to 45°C.

Sterilization: Not specified.

Use: Cultivation of *B. influenzae.*

Variants: Park, Williams and Krumwiede prepared a similar medium as follows:

(1) Remove the corpuscles from sterile defibrinated human or rabbit blood by centrifugation.
(2) Discard the supernatant fluid by pipetting off.
(3) Make up the original volume of (1) by the addition of sterile infusion broth.
(4) Prepare a 2.0% solution of neutral sodium oleate. (Kahlbaum's preferred.)
(5) Sterilize (4) in the autoclave.
(6) To 94.0 cc. of hot 2.0% nutrient agar (vitamin agar, see variant (e) medium 1863 is recommended) add 5.0 cc. of (5) and add 1.0 cc. of (3) under aseptic conditions.

References: Harvey (1921–22 p. 87), Park, Williams and Krumwiede (1924 p. 130).

1859. Brown and Orcutt's Blood Cell Agar

Constituents:

1. Veal infusion agar.
2. Blood corpuscles.

Preparation:

(1) Prepare veal infusion agar.
(2) Defibrinate blood and wash repeatedly with sterile physiological salt solution.
(3) Add some of the washed blood corpuscles to melted (1), (amounts not given) and pour into plates.

Sterilization: Not specified.

Use: To determine food requirements for *Bacillus pyogenes.* Authors reported that this medium supported the growth of *Bacillus pyogenes* nearly as well as did blood agar.

Variants: The authors prepared a similar medium as follows:

(1) Prepare veal infusion agar.
(2) Defibrinate blood and wash repeatedly with sterile physiological salt solution.
(3) Lake the washed corpuscles with distilled water and remove the corpuscle stroma by centrifugation. (Centrifuge the laked blood corpuscles until the supernatant hemoglobin solution no longer gives a clouding reaction with salt.)
(4) Wash the corpuscle stroma repeatedly in sterile distilled water until no visible trace of hemoglobin remains.
(5) Add the stroma suspension to melted agar (amount not given) and pour into plates.

This medium was inferior to blood cell medium above.

Reference: Brown and Orcutt (1920 pp. 222–223).

1860. Behren's Blood Cell Agar

Constituents:

1. Water	1000.0 cc.
2. Chopped beef	125.0 g.
3. Peptone	20.0 g.
4. NaCl	5.0 g.
5. $CaCl_2$	0.1 g.
6. N/1 Na_2CO_3 solution	10.0 cc.
7. Agar	20.0 g.
8. Red blood cells from 2000.0 cc. Rabbit blood	

Preparation:

(1) Digest chopped beef in 250.0 cc. water over night in the cold, or for one hour at 55°C.
(2) Strain (1), boil the infusion and filter
(3) Dialyze the filtrate in a large collodium sac against running distilled water for 24 to 48 hours.
(4) Dilute (3) to 1000.0 cc.
(5) Dissolve 3, 4, 5, 6 and 7 in (4).
(6) Distribute in test tubes in 1.0 cc. lots.
(7) Centrifuge defibrinated rabbit blood and draw off serum.
(8) Dilute the red blood cells, freed from serum, with 0.5% NaCl solution to the original blood volume.
(9) Shortly before use melt the desired tubes of sterile agar, cool to 60°C. and add two volumes of (8) to each tube.
(10) Mix well and solidify in slanting position.

Sterilization: Sterilize (6) in autoclave by heating to 105–108° for 15 minutes.

Use: Special culture medium for *Trypanosoma Brucei.*

Reference: Behrens (1914 p. 29).

1861. Sacquépée and Delater's Egg Albumin Infusion Agar

Constituents:

1. Distilled water	
2. Egg white	
3. Meat	500.0 g.
4. Peptone	10.0 g.
5. NaCl	5.0 g.
6. Agar	30.0 g.

Preparation:

(1) Place the whites of two eggs in a graduated glass cylinder.
(2) Shake constantly and add little by little three times the volume of distilled water.
(3) Make alkaline by the addition of about 0.5 cc. of a 10.0% soda solution for each 100.0 cc. of (2).
(4) Shake well.
(5) Heat in the autoclave at 115° for 15 minutes.
(6) Macerate 500.0 g. of meat in a liter of water.
(7) Allow to stand in the cold for 24 hours and then bring slowly to boil.
(8) Filter.
(9) Dissolve 10.0 g. peptone, 5.0 g. NaCl in (8).
(10) Dissolve 30.0 g. agar in (9).
(11) Exactly neutralize to litmus and then add 9.0 cc. of a 10.0% soda solution.
(12) Clarify by the addition of an egg white to the agar at 60°C. Heat in the autoclave and filter thru paper.
(13) Mix (5) and (12). Approximately one part (5) to five parts (12).
(14) Distribute as desired.

Sterilization: Sterilize for 15 minutes at 112°C.

Use: Cultivation of Meningococci, *Micrococcus catharralis* and *Diplococcus flavus*.

References: Sacquépée and Delater (1914 p. 224), Dopter and Sacquépée (1921 p. 139).

1862. Emile-Weil Egg Yolk Infusion Agar

Constituents:

1.	Distilled water	250.0 cc.
2.	Sea water	750.0 cc.
3.	Veal	500.0 g.
4.	Glycerol	40.0 g.
5.	Glucose	8.0 g.
6.	Peptone	10.0 g.
7.	Agar	20.0 g.
8.	Egg yolk	250.0 cc.

Preparation:

(1) Prepare infusion broth from 500.0 g. veal, 250.0 cc. distilled water and 750.0 cc. sea water. Details of method not given.

(2) Make distinctly alkaline.

(3) Add and dissolve 4, 5, 6 and 7 in (2).

(4) Add to cool agar, one part egg yolk to four parts agar. (Not clearly stated if the agar is in a liquid or solid state.)

Sterilization: Not specified.

Use: Cultivation of *B. leprae*.

Reference: Emile-Weil (1905 p. 799).

1863. Huntoon's Hormone Agar

Constituents:

1.	Water	1000.0 cc.
2.	Beef, fresh heart or steak	500.0 g.
3.	Peptone (Bacto)	10.0 g.
4.	Agar (Bacto) or soaked thread	16.0 g.
5.	Salt	5.0 g.
6.	Whole egg	1

Preparation:

(1) Chop 2 and mix 1, 2, 3, 4, 5 and 6. Place in an enameled ware vessel or a large coffee pot.

(2) Heat over a free flame with constant stirring until the red color of the meat infusion changes to brown at a temperature of about 68°C. Do not go beyond this temperature.

(3) Adjust to slightly alkaline to litmus with N/1 NaOH and then add 1.0 cc. per liter of medium.

(4) Cover the vessel and place in an Arnold sterilizer or in a water bath at 100°C. for one hour.

(5) Remove the vessel from the sterilizer and separate with a glass rod, the firm clot which has formed from the side of the vessel.

(6) Return to the Arnold sterilizer at 100°C. for 1½ hours.

(7) Remove the vessel and allow to stand at room temperature for about 10 minutes in a slightly inclined position.

(8) Pipette off the fluid portion or decant. If it is poured thru a fine wire sieve, many of the fine pieces of meat clot may be caught. (Avoid filtering thru cheese cloth, cotton or other adsorptive materials.)

(9) Allow (8) to stand in tall cylinders for 15 to 20 minutes until the fat present has risen to the surface and removed. The medium may be further cleared by filtering thru glass wool, asbestos wool, sedimentation or centrifugation.

(10) Tube in 10.0 cc. lots.

Sterilization: Sterilize intermittently in the steamer.

Use: Substitute for media containing serous fluids. Author reported that the medium had a growth value 10 times as great as standard agar and was at least as good as the average grade serum agar.

Variants:

(a) Huntoon prepared a semi-solid hormone agar as indicated above but using only 5.0 g. agar instead of 16.0 g. Huntoon reported that this medium was as good as average grade serum agar, can be employed as stab cultures, and very useful both for anaerobic and aerobic cultures. It is especially suitable for preservation of stock cultures.

(b) Huntoon added 5.0% glycerol to the medium just before tubing and reported that after ten days growth of tubercle bacilli almost as heavy as in Dorset's egg medium.

(c) Harvey gave the following method of preparation:

  (1) Prepare finely minced ox heart 500.0 g.; 10.0 g. peptone, sodium chloride 5.0 g., contents of one egg; prepared agar (see medium 1401) 16.0 g., water 1000.0 cc.

(2) Heat at a temperature not exceeding 68°C. until the red color of the mixture turns brown.
(3) Make faintly alkaline to litmus.
(4) Add after this alkalinization 1.0 cc. N/1 sodium hydroxide per liter.
(5) Steam 60 minutes.
(6) Separate the clot formed from the sides of the vessel.
(7) Steam 90 minutes.
(8) Allow to stand at room temperature 10 minutes.
(9) Pipette off the fluid and place in a tall cylinder.
(10) Leave 20 minutes.
(11) Skim off the fat from the surface.
(12) Distribute into test tubes.
(13) Sterilize at 100°C. for three days.

Harvey reported that the medium is not filtered at any stage thru any cloth, filter paper or cotton wool. If filtration is needed it should be thru glass wool. A less amount of agar, 2. g., 0.5% instead of 1.6% will give a medium in which, if sealed and kept in the incubator, meningococcus and gonococcus sown by the stab culture method will live for two or three months.

(d) Torrey and Buckell called the following medium a semi solid agar, but did not mention the addition of agar. Ascitic fluid may be used to advantage in rejuvenating delicate strains of gonococci or recovery of old stock strains. Also in primary fishing of gonococci colonies.

(1) Mix 500.0 g. of finely chopped fresh beef heart and one whole egg with water.
(2) Place in a double boiler over a free flame and maintain at 60°C. for 5 minutes. Stir constantly.
(3) Add 10.0 g. peptone and 5.0 g. NaCl and raise temperature until the mixture assumes a brownish color.
(4) Adjust reaction to slightly alkaline to litmus using 10.0% Na_2CO_3 solution.
(5) Place (4) in flask or preferably in a coffee pot and heat in Arnold steam sterilizer for an hour.
(6) Separate clot from the sides of the receptacle and replace in the sterilizer for another hour.
(7) Clear by centrifugation, or by straining thru wire mesh and then thru glass wool. Or the meat residue can be deposited on the glass wool in a funnel and the fluid be allowed to percolate thru it several times. Never use cotton or any other adsorptive materials to clarify.
(8) Adjust reaction to pH = 6.8.
(9) Reheat and tube in 7.0 cc. lots.
(10) Sterilize in Arnold sterilizer.
(11) If ascitic fluid is to be used, add 1.0 cc. to each test tube.

(e) Park, Williams and Krumwiede prepared a similar vitamin agar for the cultivation of meningococci as follows:

(1) Add 500.0 g. tap water to 500.0 g of chopped fat and tendon free beef heart.
(2) Add 15.0 g. of peptone, 5.0 g. NaCl, and one well beaten egg to (1).
(3) Heat in a water bath, double boiler or over the open flame, stirring constantly until the color changes to brown (at about 68° to 70°C.).
(4) Strain thru a wire sieve or wire gauze. Do not use cheese cloth, cotton or paper.
(5) Dissolve 15.0 g. of agar in 500.0 cc. tap water.
(6) Cool (5) to 70°C.
(7) Mix (4) and (6).
(8) Adjust the reaction to +0.2 phenolphthalein or to about pH = 7.4.
(9) Heat in an autoclave at 15 pounds pressure for 30 minutes.
(10) Remove the kettle carefully and set aside for sedimentation to take place or run in Sharples centrifuge.
(11) If the sedimentation method is used, turn the solid agar out of the kettle in mould form and trim off the layer of sediment and discard.
(12) Melt.
(13) Tube.

(14) Sterilize in an autoclave at 15 pounds for 30 minutes. This medium was recommended by the authors to be used in Avery's Oleate Blood Cell Agar, see medium 1858.

References: Huntoon (1918 pp. 170, 172), Harvey (1921–22 p. 120), Torrey and Buckell (1922 p. 127), Park, Williams and Krumwiede (1924 p. 126).

1864. Harvey's Alkaline Egg Agar

Constituents:

1. Water	500.0 cc.
2. Whites of 2 eggs	
3. Yolk of 1 egg	
4. N/1 NaOH	6.0 cc.
5. Infusion agar	5000.0 cc.

Preparation:

(1) Mix the whites of two eggs, the yolk of one egg and 6.0 cc. of normal NaOH.
(2) Add 500.0 cc. of water.
(3) Heat very slowly to 95°C.
(4) Keep at 95°C. for 60 minutes.
(5) Filter thru cotton wool.
(6) Distribute into flasks.
(7) When ready for use mix 10 parts sterile melted infusion agar, cooled to 45°C. (see variant (v) medium 1661 for preparation) with one part sterile (6) heated at 45°C.

Sterilization: Sterilize (6) in the autoclave.

Use: General culture medium.

Variants:

(a) Harvey reported that a clearer medium was obtained if the whites of two eggs were mixed with 4.0 cc. of normal NaOH and 330.0 cc. of water instead of the quantities given above.
(b) Harvey prepared a similar medium as follows:
  (1) Mix the whites of two eggs, yolk of one egg and 6.0 cc. of normal NaOH.
  (2) Add 500.0 cc. of water.
  (3) Heat very slowly to 90°C.
  (4) Distribute into flasks.
  (5) Sterilize (4) in the autoclave.
  (6) When ready for use, mix one part (5) heated to 45°C. and ten parts sterile melted infusion agar cooled to 45°C. (see variant (v) medium 1661 for preparation).

Reference: Harvey (1921–22 p. 86).

1865. Meyer's Basal Mollusk Infusion Agar

Constituents:

1. Tap Water	1000.0 cc.
2. Mollusks	500.0 g.
3. Peptone	10.0 g.
4. Agar	20.0 g.
5. Egg	2

Preparation:

(1) Place 500.0 g. of finely chopped mollusks (or beef heart), two whole eggs and 1000.0 cc. of tap water in a double boiler over a free flame and infuse at 60°C. with constant stirring for 5 minutes.
(2) Add 20.0 g. of flaked agar and 10.0 g. peptone (if desired) and raise the temperature until the mixture assumes a brownish color.
(3) Make slightly alkaline, using a 10.0% Na_2CO_3 solution.
(4) Heat in the steam sterilizer for one hour.
(5) Separate the clot from the sides of the container and place in the sterilizer for another hour.
(6) Clarify by straining thru glass wool.
(7) Adjust to pH = 7.2.
(8) Tube or flask.

Sterilization: Sterilize the fractional method at 100°C. in flowing steam.

Use: Cultivation of *Cyclostoma elegans* and other organisms living in symbiosis with mollusks.

Added nutrients:

(a) The author used the basal medium without additional material.
(b) The author added 10% defibrinated rabbit blood to the melted agar just before use.
(c) The author added 1.0 to 2.0% of filtered *Helix pomatia* or *Cyclostoma* infusion prepared as follows:
  (1) Grind 10 to 20 large sterilized Cyclostomas in a sand mortar.
  (2) Mix (1) with an equal weight of Ringer's solution. The weight of (1) to be as weight of tissue and not of sand and tissue.
  (3) Shake for ten minutes.
  (4) Centrifuge at low speed.
  (5) Remove the supernatant fluid and mix with the hemolymph of about 20 to 40 mollusks.
  (6) Pass thru a diatomatous filter, or sterilize by the addition of chloro-

form and preserve in the ice chest for four weeks or sterilize in flowing steam at 100°C. for 30 minutes.

Reference: Meyer (1925 p. 47).

1866. Faroy and Chavaillon's Egg Albumin Serum Agar

Constituents:

1. Infusion agar	
2. Serum	100.0 cc.
3. Egg white	20.0 cc.

Preparation:

(1) Add 100.0 cc. of horse serum obtained under aseptic conditions to a medium sized sterile flask containing glass beads.

(2) Add 20.0 cc. of egg white to (1). Remove the egg white by means of a sterile pipette, and add to (1) under aseptic conditions.

(3) Shake the flask vigorously for 5 to 10 minutes.

(4) Prepare a 2.5% infusion agar.

(5) Exactly neutralize (4) and add 0.2 cc. of a 10.0% soda solution per liter of agar.

(6) Mix one part (3) with 3 parts (5), melted and cooled to 50°C.

(7) Distribute as desired.

Sterilization: Not specified.

Use: Cultivation of meningococci.

Variants: Authors suggested that sugars may be added if desired,

Reference: Faroy and Chavaillon (1915 p. 455).

1867. Huntoon's Hormone Blood Agar

Constituents:

1. Water	1000.0 cc.
2. Beef heart or steak (fresh)	500.0 g.
3. Peptone (Bacto)	10.0 g.
4. Agar	16.0 g.
5. Salt	5.0 g.
6. Whole egg (one)	
7. Blood, defibrinated	

Preparation:

(1) Chop 2 and then mix with 1, 3, 4, 5 and 6. Place in an enamel ware vessel or a large coffee pot.

(2) Heat over a free flame with constant stirring until the red color of the meat infusion changes to brown at a temperature of about 68°C. Do not go beyond this temperature.

(3) Adjust to slightly alkaline to litmus with N/1 NaOH and then add 1.0 cc. per liter of medium.

(4) Cover the vessel and place in an Arnold sterilizer or in a water bath at 100° for one hour

(5) Remove the vessel from the sterilizer and separate with a glass rod the firm clot, which has formed, from the side of the vessel.

(6) Return to the Arnold sterilizer and heat at 100° for 1½ hours.

(7) Remove the vessel and allow to stand at room temperature for about ten minutes in a slightly inclined position.

(8) Pipette off the fluid portion or decant. If it is poured thru a fine wire sieve, many of the fine pieces of meat clot may be caught. (Avoid filtering thru cheese cloth, cotton or other adsorptive materials.)

(9) Allow (8) to stand in tall cylinders for 15 to 20 minutes until the fat present has risen to the surface and removed. The medium may be further cleared by filtering thru glass wool, asbestos wool, sedimentation or centrifugation.

(10) Tube in 10.0 cc. lots.

(11) Add a small amount (1.0 to 100.0 cc.) of defibrinated blood (just enough to give a pinkish tinge to the poured plate) to each tube of sterile (10).

Sterilization: Sterilize (10) intermittently in the steamer.

Use: To cultivate highly parasitic organisms. Author reported the medium especially adapted to the cultivation of the meningococci. The medium is superior to glucose ascitic agar.

Variants: Huntoon reported better results were obtained using laked blood instead of defibrinated.

Reference: Huntoon (1918 p. 171).

1868. Torrey and Buckell's Ascitic Fluid Egg Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Beef heart	500.0 g.
3. Whole egg	1
4. Peptone (Difco)	15.0 g.

5 Agar (Flaked)	18.0 g.
6 Ascitic fluid	500.0 cc.
7. Methyl violet	

Preparation:

(1) Place beef heart (preferably directly from slaughter house) and one whole egg in water.

(2) Place (1) in a double boiler over a free flame and maintain at 60°C. for 5 minutes with constant stirring.

(3) Add 4 and 5 and raise temperature until the mixture assumes a brownish color.

(4) Adjust to slightly alkaline to litmus using 10.0% solution of Na_2CO_3.

(5) Place (4) in flask, or preferably in a coffee pot and heat at 100°C. in Arnold steam sterilizer for one hour.

(6) Separate clot from sides of the receptacle and replace in sterilizer for another hour.

(7) Clear by centrifuging or by straining thru a fine wire mesh and then thru glass wool. (Do not use cotton cloth or any other material with absorbent properties.) A clear medium may also be obtained by depositing the meat residue on glass wool in a funnel and the fluid portion allowed to percolate thru.

(8) Adjust reaction to pH = 6.8.

(9) Heat and tube in 10.0 cc. lots.

(10) Add 5.0 cc. ascitic fluid to each tube of sterile melted medium just before pouring.

Sterilization: Sterilize (9) in autoclave at 12 pounds pressure for 10 minutes but preferably at 100°C. in flowing steam using the fractional method.

Use: Isolation of gonococcus. Authors reported that colonies after 24 hours were about 1.0 mm. in diameter. May show a smoky tinge by transmitted light. May show heaped up or flat centers. The edges may be very thin and slightly serrated or may be raised well defined and smooth.

Variants: 1.0 cc. of a 1:100,000 dilution of methyl violet in distilled water may be added to each tube containing 10.0 cc. of the medium and 5.0 cc. of the ascitic fluid.

Reference: Torrey and Buckell (1922 p. 126).

1869. Williams-Burdick's Modified Petroff's Egg Infusion Agar (Roddy)

Constituents:

1. Distilled water	
2. Eggs	
3. Glycerol	
4. Veal	500.0 g.
5. Gentian violet (1.0% soln.)	1.0 cc.
6. NaCl	5.0 g.
7. Agar	15.0 g.

Preparation:

(1) Dilute egg whites obtained under aseptic conditions with 10 parts distilled water. Shake thoroly.

(2) To clear, pass the fluid thru a thin layer of cotton and then heat to 100°C.

(3) Filter thru paper.

(4) Dilute egg yolk, obtained under aseptic conditions with 10 parts distilled water. Mix well.

(5) Clarify the cloudy emulsion by the addition of normal NaOH. Too much NaOH is harmful. The emulsion should be slightly turbid. Usually 1.0 cc. of normal NaOH is required for each 100.0 cc. of the emulsion.

(6) Heat to 100°C. and filter.

(7) Cover 500.0 g. of finely chopped lean veal with one liter of water containing 15.0% glycerol.

(8) Infuse for 24 hours and filter.

(9) Add 5.0 g. NaCl to the filtrate of (8) and heat to boiling.

(10) Filter.

(11) Adjust the reaction to +1.0% alkaline.

(12) Place 300.0 cc. of (3) in a liter flask.

(13) Place 300.0 cc. of (6) in another flask.

(14) Place 400.0 cc. of (11) and 15.0 g. of agar in a third flask.

(15) Remove (14) from the sterilizer and while hot add 1.0 cc. of a 1.0% alcoholic solution of gentian violet to the infusion agar.

(16) Pour the contents of the infusion agar gentian violet flask into the flask containing the egg white, and then the egg yolk is added.

(17) Pour from one flask to another to insure thoro mixing.

(18) Tube and slant.
(19) Leave the tubes in a slanted position for 72 hours at room temperature until the contents have well set. Then seal the tubes with cork and paraffin.

Sterilization: See under preparation.

Use: Cultivation of *B. tuberculosis*.

Reference: Roddy (1917 p. 46).

1870. Chapin's Egg Yolk Urine Agar

Constituents:

1. Meat infusion	1000.0 cc.
2. Urine	200.0 cc.
3. Peptone	5.0 g.
4. N/1 H_2SO_4 (1.2%)	12.0 cc.
5. Agar to solidify	
6. Pea flour (0.5%)	5.0 g.
7. Glucose (0.5%)	5.0 g.
8. Egg yolk (5.0%)	50.0 cc.

Preparation:

(1) Prepare meat infusion.
(2) Heat to remove coagulated proteid.
(3) Add 2, 3, and 4 (a normal dilution of H_2SO_4, 1.2%).
(4) Divide into 5 flasks and add varying amounts of N/1 NaOH, 0.0, 1.2, 2.3, 3.7 and 4.7% respectively having the following reactions: 3.4, 2.6, 1.8, 0.5 and alkaline.
(5) Add a sufficient quantity of agar, 0.5% of 6 and 0.5% of 7.
(6) Add 5.0% egg yolk to the cooled medium just before pouring the plates.

Sterilization: Method not specified.

Use: To study influence of CO_2 and reaction of medium on cultivation of gonococcus. The author incubated the cultures in a 10.0% CO_2 atmosphere. He reported good growth in the medium containing 4.7% NaOH the reaction being alkaline, and also in media whose reactions were +2.6 and 1.8. A lighted candle left in the jar beside the plates at the time of sealing the chamber gave a favorable atmosphere.

Reference: Chapin (1918 p. 342).

1871. Harvey's Sucrose Egg Agar

Constituents:

1. Sucrose (1.0%) Agar	150.0 cc.
2. Water	10.0 cc.
3. Na_2CO_3 (6.5% Solution)	10.0 cc.
4. Neutral red (0.5% soln.) (1.0%)	1.8 cc.
5. Egg	10.0 cc.

Preparation:

(1) Mix the contents of one egg with an equal quantity of water.
(2) Add to this mixture an equal amount of 6.5% anhydrous Na_2CO_3.
(3) Add 1 volume of the sterile mixture to 5 volumes neutral 1.0% sucrose agar.
(4) Add to the mixture while melted 1.0% freshly prepared 0.5% neutral red.
(5) Slope or pour on plates.

Sterilization: Sterilize (2) for one hour at 100°C. Method of sterilization of 1.0% sucrose agar not given.

Use: Isolation of cholera vibrio. Harvey reported that the medium had orange tint and *V. cholerae* colonies had deep red centers.

Reference: Harvey (1921–22 p. 86).

1872. Leboeuf's Egg White Liver Infusion Agar

Constituents:

1. Water	1000.0 cc.
2. Liver, horse or beef	500.0 g.
3. Peptone (Chapoteaut)	20.0 g.
4. Egg white	
5. Starch, potato	
6. Agar	

Preparation:

(1) Boil 500.0 g. of finely chopped horse or beef liver in 1000.0 cc. of water slowly for 90 minutes.
(2) Filter thru paper and make up the filtrate to the original volume.
(3) Add 20.0 g. of Chapoteaut's peptone and heat to boiling.
(4) Neutralize to litmus.
(5) Filter thru paper.
(6) Mix one part egg white with ten parts distilled water.
(7) Boil (6) and filter.
(8) Mix 100.0 cc. of sterile (7) with 1000.0 cc. of (5), and add 5.0 g. potato starch.
(9) Readjust the reaction to neutral if necessary.
(10) Add 20.0 g. of agar per 1000.0 cc. of (9) and autoclave at 115°C. for 35 minutes.
(11) Tube.

Sterilization: Sterilize (7) by heating in the autoclave at 120°C. for 20 minutes.
Use: Cultivation of gonococci.
Reference: Leboeuf (1924 p. 768).

1873. Besson's Gelatin Infusion Agar

Constituents:

1. Infusion broth	1000.0 cc.
2. Gelatin	80.0 g.
3. Agar	5.0 g.

Preparation:
(1) Dissolve 80.0 g. of gelatin in 1000.0 cc. of infusion broth.
(2) Neutralize.
(3) Dissolve 5.0 g. of agar in (2).
(4) Allow to cool to 55 or 50°C.
(5) Beat the white of an egg in 100.0 cc. of water and add to (4).
(6) Mix well.
(7) Autoclave at 115°C. for 20 minutes.
(8) Filter thru a moistened Chardin filter paper using a hot water funnel.
(9) Tube.

Sterilization: Sterilize at 115° for 20 minutes.
Use: General culture medium.
Variants: Author reported that 50.0 g. of gelatin and 8.0 g. of agar may be used instead of the amounts indicated.
Reference: Besson (1920 p. 43).

1874. Frothingham's Gelatin Infusion Agar

Constituents:

1. Infusion broth	1000.0 cc.
2, Agar	7.5 g.
3. Gelatin	50.0 g.

Preparation:
(1) Dissolve 7.5 g. agar in 1000.0 cc. of infusion broth. (A 0.5% solution of Liebig's meat extract solution may be used.)
(2) Dissolve 50.0 g. of gelatin in (1).

Sterilization: Method not given.
Use: General culture medium and to maintain stock cultures of streptococci, pneumococci and other pathogens. North reported that the addition of 1.0% glucose made the growth more favorable at times, but reduced vitality.
Variants:
(a) North gave the following method of preparation:
  (1) Extract one pound of lean chopped beef or veal in 500.0 cc. of water for 18 hours.
  (2) Add and dissolve in this infusion, made up to 1 liter with water, 10.0 g. agar, 20.0 g. gelatin, 20.0 g. Witte peptone and 5.0 g. salt.
  (3) Adjust to the neutral point using phenolphthalein as an indicator.
  (4) Tube.
(b) Dopter and Sacquépée prepared the medium as follows:
  (1) Add 1000.0 cc. of water to 500.0 g. of finely chopped fat and tendon free beef.
  (2) Allow to stand in the ice box for 12 hours, or heat at 50 to 55° for 30 minutes.
  (3) Heat slowly to boiling.
  (4) Boil slowly for 10 minutes, stirring constantly.
  (5) Strain thru a cloth.
  (6) Dissolve 80.0 g. gelatin, 5.0 g. NaCl and 20.0 g. peptone in (5).
  (7) Neutralize.
  (8) Dissolve 5.0 g. agar in (7).
  (9) Clarify by the addition of egg white.
  (10) Autoclave.
  (11) Filter.
  (12) Distribute
  (13) Sterilize (method not given).
(c) Guarniari (Abbott) gave the following method of preparation:
  (1) Dissolve 3.0 to 4.0 g. agar separately in about 100.0 cc. of water while the other materials are being prepared.
  (2) Prepare meat infusion.
  (3) Dissolve 5.0 g. NaCl, 25.0 to 30.0 g. peptone and 40.0 to 60.0 g. gelatin in (2).
  (4) Mix (3) and (1).
  (5) Sterilization not specified.
(d) Harvey solidified infusion broth, see variant (bb) medium 779 by the addition of 10.0 or 12.0% gelatin and 0.5% agar.
(e) Park, Williams and Krumwiede gave the following method of preparation:
  (1) Prepare a meat infusion using 1.0 pound of meat to 500.0 cc. of water (Method not given).

(2) Dissolve 20.0 g. gelatin and 20.0 g. peptone in warmed (1).
(3) Dissolve 10.0 g. of agar in 500.0 cc. of water and cool to below 50°C.
(4) Mix (2) and (3).
(5) Adjust the reaction.
(6) Heat to coagulate the albumin and clear the medium.
(7) Readjust the reaction and reheat if necessary.
(8) Filter.
(9) Sterilization not specified.

References: Frothingham (1895 p. 53), North (1909 p. 361), Tanner (1919 p. 51), Dopter and Sacquépée (1921 p. 128), Abbott (1921 p. 144), Harvey (1921–22 p. 71), Stitt (1923 p. 39), Park, Williams and Krumwiede (1924 p. 118).

1875. Krause's Gelatin Urea Agar

Constituents:

1. Infusion agar (3.0%)	1000.0 cc.
2. Infusion gelatin (20.0%)	2000.0 cc.
3. Urea (2.5%)	75.0 g.

Preparation:
(1) Prepare a 3.0% meat infusion agar.
(2) Prepare a 20.0% meat infusion gelatin.
(3) The NaCl content of (1) and (2) should be about 0.7 to 0.8%. The reaction is not adjusted.
(4) Mix one part (1) with two parts (2).
(5) Adjust the reaction to 0.27 to 0.3% lactic acid.
(6) Dissolve 2.5% urea in the least amount of water and thoroly mix with (5).
(7) Distribute into sterile tubes.
(8) Before use, melt sterile (7) and pour into sterile plates.

Sterilization: Steam (7) for 15 minutes in flowing steam.

Use: Detection of typhoid bacilli. Author reported that typhoid colonies had a nucleus surrounded by thread-like appendages, they were grey in color when young and brown when old.

Reference: Krause (1902 p. 94).

1876. MacNeal and Kerr's Gelatin Agar

Constituents:

1. Infusion broth	1000.0 cc.
2. Agar	7.5 g.
3. Gelatin	50.0 g.
4. Blood serum (Sterile)	500.0 cc.

Preparation:
(1) Prepare usual infusion broth.
(2) Dissolve 2 and 3 in (1) by heat and tube.
(3) Cool sterile (2) to 45°C.
(4) Add 4. (½ volume of 4 to (3)).
(5) Inoculate while still a liquid.
(6) Mix medium and solidify by immersing in cold water.

Sterilization: Sterilize (2). (Method not given).

Use: Culture medium for *Bacillus abortus*. The authors reported a growth zone 5 mm. beneath the surface of the medium and extending down for 1.0 to 1.5 cm.

Reference: MacNeal and Kerr (1910 p. 469).

1877. Supplee's Nutrose Gelatin Agar (Ayres and Johnson)

Constituents:

1. Meat infusion	1000.0 cc.
2. Gelatin	20.0 g.
3. Agar	10.0 g.
4. Peptone	20.0 g.
5. Nutrose	5.0 g.

Preparation:
(1) Prepare meat infusion.
(2) Dissolve 2, 3, 4 and 5 in (1).
(3) Adjust to neutral with phenolphthalein.

Sterilization: Not specified.

Use: Used especially in carrying stock cultures of streptococci and similar organisms.

Reference: Ayers and Johnson (1924 p. 111).

1878. Kinsella, Brown and Garcia's Nutrose Gelatin Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Beef	500.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Agar	5.0 g.
6. Gelatin	50.0 g.
7. Nutrose	10.0 g.

Preparation:
(1) Mix finely chopped beef in water and set in the ice chest for 24 hours.
(2) Strain thru linen towel.
(3) Add and dissolve 3 and 4 in (2).
(4) Boil to clear and filter thru filter paper.

(5) Add 5, 6 and 7 to (4).
(6) Boil over saturated solution of NaCl in a double boiler for 45 minutes.
(7) Strain thru absorbent cotton.
(8) Titrate to pH = 7.3.
(9) Tube.

Sterilization: Sterilize in autoclave for 20 minutes.

Use: To maintain cultures of gonococci. Authors reported that growth started as a film on the surface and radiated from point of inoculation. The gonococci survived on this medium for from 3 to 4 weeks.

Reference: Kinsella, Brown and Garcia (1923 p. 4).

1879. Ayers and Johnson's Casein Gelatin Agar

Constituents:

1. Distilled water	500.0 cc.
2. Meat infusion broth	500.0 cc.
3. Peptone (Parke-Davis)	10.0 g.
4. Gelatin (Difco)	10.0 g.
5. Casein (pure, prepared according to Hammarsten)	5.0 g.
6. Glucose	0.5 g.
7. $Na_2HPO_4 \cdot 2H_2O$ (Sorrenson's phosphate)	4.0 g.
8. Sodium citrate	3.0 g.
9. Agar	7.5 g.

Preparation:

(1) Prepare meat infusion broth.
(2) Add 10.0 g. peptone (Parke-Davis) and 2.0 g. $Na_2HPO_4 \cdot 2H_2O$ (Sorrenson's phosphate.) Heat until solution takes place.
(3) Adjust the reaction to pH = 7.8.
(4) Dissolve 5.0 g. pure casein (prepared according to Hammarsten) and 2.0 g. $Na_2HPO_4 \cdot 2H_2O$ (Sorrenson's phosphate) in 150.0 cc. distilled water by heating.
(5) Mix (4) and (3).
(6) Add 10.0 g. of gelatin (Difco) to (5).
(7) Heat (6) in the autoclave at 15 pounds for 10 minutes.
(8) Add 0.5 g. glucose.
(9) Reaction should be pH = 7.6.
(10) Filter thru paper.
(11) Prepare 250.0 cc. of 3.0% agar (Not specified if the agar is to be dissolved in meat infusion or water).
(12) Filter (11) thru cotton and dissolve 3.0 g. of sodium citrate in the melted agar.
(13) Mix (12) and (10) and make up to 1000.0 cc. by adding distilled water.
(14) Tube.
(15) Final pH = 7.5.

Sterilization: Sterilize for 20 minutes at 15 pounds in the autoclave. (A precipitate may be formed. It will settle out or be absorbed in a short time. In either case the medium is not effected).

Use: To carry stock cultures of streptococci, *Diplococcus pneumoniae*, *Hemophilus pertussis*, *Pasteurella bovis*, *Erysipelothrix porci*.

Reference: Ayares and Johnson (1924 p. 112).

1880. Frazier's Gelatin Agar

Constituents:

1. Distilled water	1000.0 cc.
2. NaCl	5.0 g.
3. KH_2PO_4	0.5 g.
4. K_2HPO_4	1.5 g.
5. Gelatin Bacto	4.0 g.
6. Peptone Bacto	0.1 g.
7. Glucose	0.05 g.
8. Beef infusion	5.0 cc.
9. Agar (3.0%)	15.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 100.0 cc. distilled water.
(2) Dissolve 4.0 g. Bacto gelatin in 400.0 cc. distilled water and add 0.05 g. glucose, 0.1 g. Bacto peptone, and 5.0 cc. of beef infusion.
(3) Mix (1) and (2) and heat in a steamer.
(4) Prepare 500.0 cc. of a 3.0% washed agar.
(5) Mix (4) and hot (3).
(6) Adjust to pH = 7.0.
(7) Tube or flask.

Sterilization: Sterilize in the autoclave.

Use: To determine gelatin decomposition. Pour plates of the medium, allow to harden, inoculate duplicate plates, on the surface of the medium, so as to form a giant colony. Following incubation for 2 or 3 days, flood one plate with a 1.0% solution of tannic acid while the duplicate plate is flooded with an acid solution of bichloride of mercury, ($HgCl_2$ 15.0 g., HCl (con.) 20.0 cc. and water 100.0 cc.). If the gelatin has been attacked, a clear zone will appear about the colony on the plate flooded with acid bichloride of mercury. The plate flooded with tannic acid presents a white to clear zone about the colony depending on the vigor with which the organism attacked the gelatin.

Reference: Frazier (1926 p. 302).

1881. Duval and Lewis' Glucose Blood Agar

Constituents:

1. Infusion agar
2. Glucose (0.5 to 1.0%)...5.0 to 10.0 g.
3. Blood, defibrinated (2.0 to 5.0%).......... 20.0 to 50.0 cc.

Preparation:

(1) Prepare beef infusion agar.

(2) Adjust reaction of (1) but with one change if possible between 0.4 and 0.8% acid to phenolphthalein (cold titration).

(3) Add glucose from 0.5 to 1.0%, also from 2.0 to 5.0% "fresh" defibrinated blood.

Sterilization: Not specified.

Use: Cultivation of pneumococci. Similar media were employed to cultivate a large variety of organisms.

Variants:

(a) Novy (Hagemeister) cultivated pathogenic trypanosomes on a medium prepared as follows:

(1) Prepare an extract of 125.0 g. beef (or horse meat) in one liter of water.

(2) Dissolve 20.0 g. agar, 20.0 g.peptone and 5.0 g. NaCl in (1).

(3) Neutralize to litmus by the addition of N/1 NaOH.

(4) Add 5.0 cc. of N/1 NaOH per liter.

(5) Dissolve 2.0% glucose in (4).

(6) Distribute in 3.0 or 4.0 cc. lots in test tubes.

(7) Add a double or triple amount of sterile defibrinated blood to each tube of sterile agar, melted and cooled to 60°C. Mix well.

(8) Solidify in a slanted position.

(9) After inoculation seal the tubes with paraffin.

(b) Bushnell used the following medium for the isolation of meningococci. Author reported that by reflected light, meningococci colonies appeared raised, somewhat convex, glistening, moist, translucent and somewhat brownish, with uniform slightly granular structure. By transmitted light they were translucent, homogeneous and showed no zone of hemolysis. Best results were obtained by using blood as fresh as possible. Laking was not necessary. If plates are warmed before use, more rapid and abundant growth of meningococci was obtained.

(1) Exact method of preparation or exact composition of 2.0% meat infusion agar not given.

(2) Dissolve 1.0 peptone in (1).

(3) Adjust to exactly neutral to phenolphthalein.

(4) Distribute in 300.0 cc. lots in 500.0 cc. flasks.

(5) Autoclave at 15 pounds for 30 minutes.

(6) Sterilize 5.0 g. glucose in 50.0% solution.

(7) Cool the melted agar to 45°C. and add the slightly warmed glucose solution and 50.0 cc. sterile defibrinated sheep's (or whole) blood.

(8) Mix by a whirling motion avoiding air bubbles.

(9) Pour in sterile plates.

(c) Hertig and Wolbach cultivated *Rickettsia melpohagi* on a medium prepared as follows:

(1) Prepare beef bouillon in the usual manner (exact procedure not given).

(2) Dissolve 20.0 g. glucose and 25.0 g. agar in (1).

(3) Method of sterilization not given.

(4) To melted (3) at 100°C. add sterile defibrinated rabbit blood in equal volume to double volume.

(5) Slant the tubes and seal with rubber caps to prevent evaporation.

References: Duval and Lewis (1905 p. 474), Hagemeister (1914 p. 228), Bushnell (1918 p. 2), Hertig and Wolbach (1924 p. 337).

1882. Torrey's Glucose Blood Agar

Constituents:

1. Distilled water............. 1000.0 cc.
2. Liver, beef................. 500.0 g.

3. Peptone	10.0 g.
4. Agar	20.0 g.
5. Glucose	10.0 g.
6. K_2HPO_4	1.0 g.
7. Blood, sterile defibrinated rabbit	100.0 cc.

Preparation:

(1) Chop 500.0 g. beef liver into small pieces and boil for 2 hours in 1000.0 cc. of distilled water in a double boiler.
(2) Filter thru flannel and cotton.
(3) Add to filtrate peptone and agar.
(4) Heat in Arnold for one hour.
(5) Adjust reaction as desired (for *B. bifidus*, +1 to phenolphthalein).
(6) Clear with eggs if necessary.
(7) Add glucose and K_2HPO_4 to clear filtrate.
(8) To each 10.0 cc. of sterile medium add about 1.0 cc. of sterile defibrinated rabbit blood just before pouring the plate.

Sterilization: Method not given.

Use: Isolation of *B. bifidus*. To isolate *B. bifidus* pour medium in petri dish. Prepare nutrient agar seeded with *B. cereus* and pour in cover of the Petri dish. Allow the liver agar to solidify and nutrient agar only partially. When nutrient agar is semi solid, invert the inoculated liver agar plate and place it in the semi solid agar. The solidifying agar forms a seal. This gives partial anaerobiosis. Author reported colonies were raised, more or less globular, opaque, 1 to 3 mm. in diameter, buff to reddish brown in color. *B. acidophilus* gave serrated edge colony—flat. To isolate *B. acidophilus* use this medium omitting blood and adjusting to +4 acid. Grow aerobically.

Variants: Harvey solidified medium 833 variant (a) by the addition of agar.

References: Torrey (1922 p. 435), Harvey (1921–22 p. 110).

1883. Erickson and Albert's Testicular Blood Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Beef testicle	500.0 g.
3. Peptone	20.0 g.
4. Glucose	5.0 g.
5. NaH_2PO_4	2.0 to 3.0 g.
6. Agar, granular	25.0 g.
7. Blood, human	5.0 to 25.0 cc.

Constituents:

(1) Remove all connective tissue from beef testicles, put thru meat grinder and infuse on ice over night.
(2) Next morning heat in a double boiler to 50°C., allow to stand an hour and then bring to the boiling point.
(3) Let stand for another hour to permit the solid particles to settle.
(4) Decant off the liquid.
(5) Dissolve 3, 4, 5 and 6 in (4) by heating over a free flame stirring constantly.
(6) Titrate using phenol red as an indicator to pH = 7.4 to 7.8 or a reaction of 0.6 to phenolphthalein.
(7) Tube and autoclave at 15 pounds for 20 minutes.
(8) Check titration.
(9) While (8) is still liquid, add human blood in proportion of 0.5 to 2.5%.

Sterilization: See step (7) above.

Use: Isolation and cultivation of gonococci. Author reported that using phenol red as an indicator primary acidity and secondary alkalinity may be determined. In a case of mixed infection, methyl violet in proportion of 1:200,000 to 500,000 inhibited the growth of staphylococci.

Variants: The authors substituted 1.0 to 5.0% defibrinated rabbit blood for human blood.

Reference: Erickson and Albert (1922 p. 277).

1884. Harvey's Lactose Blood Agar

Constituents:

1. Infusion agar	1000.0 cc.
2. Lactose	30.0 g.
3. Blood, sterile defibrinated human	
4. Rosolic acid (1.0%)	

Preparation:

(1) See variant (v) Medium 1661 for preparation of infusion agar.
(2) Mix 0.3 g. lactose with each 10.0 cc. of (1).
(3) Raise slowly to boiling water temperature.
(4) Cool to 45°C.

(5) Add 1.0 cc. defibrinated sterile human blood at 45°C., and 3.0 cc. 1.0% alcoholic rosolic acid at 45°C.
(6) Prepare plates.
(7) Keep two days before use.

Sterilization: Not specified.
Use: Cultivation of colon-typhoid group.
Reference: Harvey (1921–22 p. 73).

1885. Harvey's Saponin Blood Agar

Constituents:
1. Infusion agar.
2. Saponin.
3. Blood, defibrinated.

Preparation:
(1) See variant (v) medium 1661 for preparation of infusion agar.
(2) Make a saturated solution of defibrinated blood in 1.0% saponin.
(3) Mix (1) and (2), amounts not given.

Sterilization: Not specified.
Use: General culture medium.
Reference: Harvey (1921–22 p. 76).

1886. Warden's Blood Veal Agar

Constituents:

1. Veal infusion	1000.0 cc.
2. Agar	20.0 g.
3. Blood, defibrinated rabbit	500.0 cc.

Preparation:
(1) Prepare salt free veal infusion broth.
(2) Dissolve agar in (1).
(3) Neutralize to phenolphthalein.
(4) Add defibrinated rabbit blood.

Sterilization: Not specified.
Use: Cultivation of gonococci.
Reference: Warden (1915 p. 426).

1887. Wherry and Ervin's Glycerol Blood Agar

Constituents:

1. Beef infusion	1000.0 cc.
2. Peptone (Witte's)	10.0 g.
3. Na_2HPO_4	5.0 g.
4. Glycerol	60.0 g.
5. Blood, defibrinated rabbit	100.0 cc.

Preparation:
(1) Prepare lean beef infusion.
(2) Dissolve 2, 3, and 4 in (1).
(3) Adjust to a reaction from +1.5 to +2.0 to phenolphthalein.
(4) Tube in 5.0 cc. lots.
(5) Add to each sterile tube of (4) 0.5 cc. of defibrinated rabbit blood.

Sterilization: Sterilize (4) in the autoclave.
Use: Cultivation of *B. tuberculosis*.
Reference: Wherry and Ervin (1918 p. 194).

1888. Elser and Huntoon's Basal Blood Infusion Agar

Just before inoculation of tube of medium 1677, cover the slanted surface with several drops of defibrinated human blood which has been allowed to age for some time in order to reduce to the minimum its initial bactericidal properties. Authors reported this medium especially adapted to the cultivation of freshly isolated cultures.

1889. North's Gelatin Blood Agar (Kligler)

Same as medium 1874 with the addition of 1.0 cc. of defibrinated rabbit blood per 10.0 cc. tube of North's gelatin agar.
Reference: Kligler (1915 p. 329).

1890. Dieudonne's Alkaline Blood Agar (Harvey)

Constituents:

1. Infusion agar	140.0 cc.
2. Blood, defibrinated ox	10.0 cc.
3. NaOH (normal solution)	10.0 cc.

Preparation:
(1) Mix equal parts defibrinated ox blood and normal NaOH solution (or 11.4% Na_2CO_3 may be used instead of NaOH).
(2) See variant (v) medium 1661 for preparation of infusion agar.
(3) Neutralize (2) to litmus.
(4) Mix 3 parts sterile (1) at 45°C. with 7 parts sterile (3) cooled to 45°C.
(5) Distribute immediately into test tubes or plates. Should be kept freely open after tubing or plating, under sterile sheets of paper, for 48 hours at 37°C., or under a bell jar containing carbon dioxide gas for 30 to 60 minutes.

Sterilization: Sterilize (1) at 100°C. on each of 3 successive days.
Use: Isolation of *V. cholerae*.
Variants: Harvey gave the following variants.
(a) (1) Mix equal parts normal NaOH and defibrinated ox blood.

(2) Steam for 60 minutes.
(3) Desiccate in vacuo over sulphuric acid or calcium chloride.
(4) Grind the residue to powder.
(5) Make for use a 10.0% solution of blood powder.
(6) When ready for use add 3 parts (5) at 45°C. to 7 parts infusion agar at 45°C.

(b) (1) Mix equal parts 12.0% crystalline Na_2CO_3 solution and defibrinated blood.
(2) Mix 3 parts (1) with 7 parts of 4.0% infusion agar.

Reference: Harvey (1921–22 pp. 73, 75).

1891. Filde's Blood Digest Agar (Kristensen)

Constituents:

1. Water	25,000.0 cc.
2. Beef	25,000.0 g.
3. Peptone	1.0%
4. NaCl	0.5%
5. Blood, defibrinated sheep or horse	

Preparation:

(1) Add 6.0 cc. of pure concentrated HCl to 150.0 cc. of a 0.9% NaCl solution, and then 50.0 cc. defibrinated horse or sheep blood and 2.0 g. Langebek's concentrated pepsin.
(2) Pour into a sterile flask and incubate at 56°C. for 5 or 6 hours.
(3) Add NaOH solution until a sample diluted with distilled water to a light brown color turns red on the addition of phenol red, without giving the alkaline color change with α-naphtholphthalein.
(4) Add 0.25% chloroform and stopper the flask with a sterile rubber and keep in a cold room.
(5) Mince 25,000.0 g. beef.
(6) Add 25 liters of water and allow to stand in the cold until the next day.
(7) Boil for a short time.
(8) Press the fluid from the meat.
(9) Add about 16 liters of water to the meat, heat to boiling once more and again press the juice from the meat.
(10) Mix the fluid from (7) and (8).
(11) Add 1.0% peptone and 0.5% NaCl.
(12) Adjust to pH about 7.8 to 7.9.
(13) Add 2.0% agar and dissolve.
(14) Filter thru cotton wool and distribute in 600.0 cc. flasks.
(15) The pH of sterile agar should be about 7.2.
(16) Add 30.0 cc. of (4) to each 600.0 cc. of melted (15) cooled to 40 to 50°C.

Sterilization: Sterilize (10) by heating in the steamer on each of 3 consecutive days.

Use: Cultivation of influenza bacilli, Haemoglobinophilic bacteria.

Reference: Kristensen (1922 p. 229).

1892. MacNeal's Blood Infusion Agar

Constituents:

1. Water	1000.0 cc.
2. Chopped beef	125.0 g.
3. Agar	20.0 g.
4. Peptone	20.0 g.
5. NaCl	5.0 g.
6. N/1 Na_2CO_3 soln.	10.0 cc.
7. Blood, naturally sterile defibrinated rabbit	2000.0 cc.

Preparation:

(1) Extract 125.0 g. beef in 1000.0 cc. distilled water.
(2) Dissolve 3, 4, 5 and 6 in (1).
(3) Tube.
(4) Add to sterile (3), cooled to about 60° two volumes of naturally sterile defibrinated rabbit's blood. Mix thoroly.
(5) Slant and solidify.

Sterilization: Sterilize (3) in the autoclave.

Use: Isolation of Nagana parasite, *Tr. Brucei* and other trypanosomes. Inoculate Nagana blood in the small amount of liquid which collects on the surface of the blood agar. Thompson cultivated trypanosomes found in gold fish (*T. danilewskyi*).

Variants:

(a) The author used 4 times as much meat (500.0 g. per liter) for succeeding cultures.
(b) Tobey used the same medium and added two volumes of defibrinated rabbit's blood. He inoculated the water of condensation with a drop of blood taken from the heart of a bird by means of a drawn-out tube pipette. The cotton plug is then cut short, moistened with mercuric chloride

and the tube covered with a rubber cap, after which it is placed at 25°C. for about one week.

(c) Behrens gave the following method of preparation:

(1) Digest 125.0 g. chopped beef in 250.0 cc. water over night in the cold, or for one hour at 55°C.
(2) Strain (1), boil the extract and filter.
(3) Dialyze the filtrate in a large collodium sac against running distilled water for 24 to 48 hours.
(4) Dilute (3) to 1000.0 cc.
(5) Dissolve 20.0 g. peptone, 5.0 g. NaCl, 0.1 g. $CaCl_2$, 10.0 cc. N/1 Na_2CO_3 solution and 20.0 g. agar in (4).
(6) Distribute in test tubes in 10.0 cc. lots.
(7) Sterilize in autoclave by heating to 105–108° for 15 minutes.
(8) Shortly before use, the desired number of agar tubes are melted in the water bath, cooled to 60°C. and two volumes of defibrinated rabbit's blood are added.
(9) Mix well and solidify in slanting position.

References: MacNeal (1904 p. 535), Tobey (1906 p. 125), Novy and MacNeal (1905 p. 265), Thompson (1908 p. 77), Behrens (1914 p. 27).

1893. Smedley's Blood Infusion Agar

Constituents:

1. Bullock heart infusion	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	10.0 g.
4. Agar	20.0 g.
5. Blood, defibrinated rabbit	1000.0 cc.

Preparation:

(1) Prepare an infusion of bullock's heart.
(2) Dissolve 1.0% peptone and 1.0% NaCl and 2.0% agar in (1).
(3) Clear in the usual way by the addition of the white of an egg.
(4) Tube.
(5) Collect blood from the heart of a rabbit by means of sterilized Pasteur bulbs and then defibrinated in sterilized bottles containing a little broken glass.
(6) Cool sterile (3) to 45°C. and add an equal volume of (5).
(7) Mix and slant.

Sterilization: Method of sterilization of (4) not given.

Use: Cultivation of *Trypanosomata Lewisi* and other trypanosomata. Other investigators cultivated a number of different organisms on similar media.

Variants:

(a) Duval (Gurd) prepared a medium as follows:

(1) Prepare a beef infusion.
(2) Dissolve 10.0 g. peptone, 20.0 g. agar and 5.0 g. NaCl in (1).
(3) Adjust reaction to 0.6% acid to phenolphthalein (hot titration).
(4) Tube.
(5) Sterilize in the autoclave.
(6) Cool to 52°C. and add a small quantity of defibrinated sterile human blood. From 4 to 7 drops of blood are added to each 6 to 10.0 cc. of agar.
(7) Shake thoroly.
(8) Slant or pour in sterile Petri dishes.

Gurd used the medium to isolate gonococci. He reported that gonococci colonies were raised worty-looking, bluish gray or almost colorless semi-transparent and round. Gave a bright crimson almost transparent medium. If agar was above 60°C. when mixed with blood, the hemoglobin was destroyed and medium assumed a dirty brown color.

(b) Duval cultivated *B. leprae* on a medium prepared as indicated. He reported that cultures without the blood and using *B. typhosus* and *Entameba coli* gave growth of *B. leprae*.

(1) Prepare nutrient infusion agar.
(2) Sterilize (Method not given).
(3) Add 1.0% human defibrinated blood to sterile (2).
(4) Slope.
(5) Inoculate the surface with pure growth cultures of *B. influenza* and meningococcus.
(6) Incubate at room temperature for 48 hours.
(7) Inoculate the slant with an emulsion prepared from leprous tissue.

(c) Hagemeister cultivated pathogenic trypanosomes in the following medium:
- (1) Infuse one pound of finely chopped beef or horse meat with one liter of water for 24 hours.
- (2) Boil for one hour.
- (3) Filter thru a towel.
- (4) Make up to 1 liter by the addition of water.
- (5) Add 10.0 g. peptone (Sicc.), 20.0 g. agar and 5.0 g. NaCl to (4).
- (6) Heat to boiling, stirring constantly.
- (7) Neutralize by the addition of N/1 NaOH using litmus as an indicator.
- (8) Add 5.0 cc. of N/1 NaOH per liter.
- (9) Heat in the autoclave at 100°C. for one hour.
- (10) Filter thru a double filter or cotton and distribute in sterile tubes.
- (11) Sterilize in the steamer for one hour on each of three successive days.
- (12) Add 2 or 3 times the volume of sterile defibrinated blood to each tube of agar, melted and cooled to 60°C. Mix well.
- (13) Solidify in a slanted position.
- (14) Seal the tubes with paraffin after inoculation.

(d) Warden isolated and cultivated gonococci on the following medium:
- (1) Dissolve 22.5 g. agar in 1000.0 cc. of salt free broth.
- (2) Neutralize to phenolphthalein.
- (3) Add 25.0 cc. of sterile defibrinated rabbit blood at 60°C. to 1000.0 cc. of sterile (2) cooled to 60°C. (The blood may be omitted.)

Warden reported that growth was better if agar was completely dry (in desiccators over H_2SO_4) before inoculation.

(e) Holman studied hemolysis by streptococci on a medium prepared as follows:
- (1) Plain infusion agar (+0.6) is sterilized in 100.0 cc. quantities in flasks.
- (2) Heat a flask of (1) in the autoclave and place in the paraffin oven at 58°C. for some time.
- (3) Cool the flask to 50°C. and thoroly mix 5.0 cc. of defibrinated human blood with the fluid agar.
- (4) Pour this mixture into petri dishes for blood agar plates on the surface of previously prepared agar.
- (5) The agar for (4) is prepared by dissolving 1.5% agar in normal saline (0.85% NaCl), filtered, tubed and sterilized.
- (6) Pour the blood agar mixture (3) on the plain agar (5) to a depth of about 2 millimeters.

He reported that it was advisable to add the serum with the red blood cells as the serum is an important aid in hemolysin production.

(f) Kohman studied the oxygen tension of meningococci on the following medium:
- (1) Prepare a 2.5% meat infusion agar.
- (2) Dissolve 10.0 g. of Bacto peptone in 1000.0 cc. of (1).
- (3) Add 40.0 cc. of human defibrinated blood to (2).

(g) Bernstein and Lowe used the following medium for the isolation of influenza bacilli:
- (1) Prepare infusion agar with a reaction of pH = 7.1.
- (2) Add gentian-violet so that the content be 1:5,000, taking a concentrated alcoholic solution of dye as unity.
- (3) Method of sterilization not given.
- (4) Add sterile defibrinated human blood to sterile melted (3).
- (5) Pour in sterile plates and store in refrigerator for use.
- (6) Streak surface with washed sputum or material from nasopharynx.

The author reported that pneumococci, streptococci and staphylococci were nearly all inhibited. If ascitic fluid be added, the gram + organisms were not inhibited by the dye.

(h) Wahl, White and Lyall cultivated influenza bacilli on a medium prepared as follows:
- (1) Prepare neutral beef infusion agar.
- (2) To every 100.0 cc. of sterile (1) add 5.0 cc. of sterile defibrinated sheep blood at 45 to 50°C.

(3) Mix thoroly and pour in plates.

(i) Bell cultivated influenza bacilli in a medium containing 4.0% defibrinated rabbit blood to a 2.0% infusion agar.

(j) Yoshida cultivated *Entamoeba tetragena* and *Entamoeba coli* from their cysts in the water of condensation of an agar containing one part defibrinated horse blood to two parts agar. The blood and agar were mixed at 60°C.

(k) Anderson and Schultz added 2.0% defibrinated blood to infusion agar and heated to 80°C. until a brown color is produced. They cultivated *Bacillus pfeifferi* on this medium.

(l) Harvey mixed one part of sterile defibrinated blood at 45°C. with two parts infusion agar (see variant (v) medium 1661).

(m) Novy-MacNeal (Harvey) cultivated leishmania on a medium prepared by mixing one part melted infusion agar at 45°C. containing 2.0% peptone (see variant (aa) medium 1375) with two parts sterile defibrinated rabbit blood at 45°C.

(n) Harvey cultivated gonococci on a medium prepared by adding 5 drops of sterile defibrinated blood at 45°C. to a tube of infusion agar (see variant (v) medium 1661) with a reaction of 0.6% acid to phenolphthalein at 45°C. Preferably infusion agar in which sodium chloride is replaced by di-sodium phosphate. The substitution of phosphate is important. The blood is sterilized by heating 8 days at 57°C. Serum may be used instead of blood.

(o) Harvey cultivated *B. influenza*, etc., on a medium prepared as follows:

(1) Boil 1.0 cc. blood with 9.0 cc. water.

(2) Allow to deposit.

(3) Add 0.5 cc. clear colorless supernatant fluid to 5.0 cc. melted infusion. (See variant (v) medium 1661.)

(p) Harvey used the following medium to cultivate *B. influenza* etc.:

(1) Mix 1.0 cc. of defibrinated rabbit or human blood with 20.0 cc. of infusion agar at 70°C. (See variant (v) medium 1661.)

(2) Raise the temperature of the mixture to boiling point over a free flame.

(3) Shake to mix.

(4) Raise to boiling point twice again.

(5) Allow to deposit.

(6) Decant the clear supernatant fluid into test tubes while the agar is still melted, or filter thru glass wool.

(7) Slope.

(q) Harvey prepared a medium as follows:

(1) Mix one part defibrinated ox blood at 70°C. with 20 parts of infusion agar at 70°C. (See variant (v) medium 1661.)

(2) Steam 45 minutes.

(3) Allow to solidify.

(4) Steam to melt the medium.

(5) Strain thru glass wool, filled to a depth of 1/8th inch into a Buchner funnel, under slightly reduced pressure.

(6) Distribute into test tubes.

(7) Sterilize at 100°C. on each of 3 successive days.

(r) Jones cultivated an organism resembling *Bacillus actinoides* from pneumonic rat lung on a medium prepared as follows:

(1) Prepare veal infusion agar.

(2) Slant.

(3) Add 0.5 cc. of defibrinated horse blood, or calf serum, to the water of condensation.

(4) Remove a small piece of lung tissue from an infected rat under aseptic conditions and push the tissue down into the tubes.

(5) Seal the tubes with sealing wax.

(s) Pitfield added 1 part sterile defibrinated blood to 5 parts sterile melted agar.

(t) Stitt prepared a N. N. N. medium for the cultivation of trypanosomes, leishmania and protozoa in the following manner:

(1) Cover 125.0 g. chopped beef with 1000.0 cc. of water and place over night in the refrigerator.

(2) Strain.
(3) Add 20.0 g. of peptone, 5.0 g. NaCl, 10.0 cc. normal Na_2CO_3 solution and 20.0 to 25.0 g. agar.
(4) Prepare as for nutrient agar and sterilize (method not given).
(5) To one part melted and cooled to 60°C. (4) add two volumes of defibrinated rabbit blood under aseptic conditions.
(6) Slant.
(7) Cover the cotton plugs with melted paraffin or use rubber stoppers to seal the tubes.

(u) Brown (Stitt) differentiated streptococci on the following medium:
(1) Prepare an agar base using 500.0 g. veal, 5.0 g. NaCl, 15.0 g. agar and 10.0 g. peptone in the same manner as for the preparation of ordinary nutrient agar.
(2) Reaction of (1) to be between +0.8 and +1.2.
(3) Tube in 12.0 cc. amounts.
(4) To prepare the blood agar melt the tubes of agar and place in a water bath at 45°C. for 15 minutes.
(5) Add 0.6 cc. defibrinated blood to each tube and mix thoroly.
(6) Inoculate.
(7) Pour into plates.

(v) Beilin (Klimmer) cultivated influenza bacilli on a medium prepared as follows:
(1) Heat 3.0% infusion agar with 1.0% peptone to boiling.
(2) Add 15.0% of sterile fresh defibrinated horse blood to boiling (1).
(3) Remove from the flame.
(4) Cool to 50 to 60°C. and mix thoroughly.
(5) Pour in plates.

(w) Schottmüller (Klimmer) cultivated gonococci, meningococci and streptococci on a medium prepared by mixing five parts liquified agar at 45°C. with two parts sterile defibrinated human blood.

(x) Novy and MacNeal (Park, Williams and Krumwiede) cultivated flagellate, trypanosomes and leishmania on a medium prepared as follows:
(1) Mix equal parts of nutrient agar and fresh defibrinated blood (rabbit or rat).
(2) Allow the medium to stiffen slanted so more of the water of condensation will settle at the bottom.
(3) Sterilization not specified.
(4) Inoculate with infected blood.

(y) Soule cultivated *Trypanosoma Lewisi* and *Leishmania tropica* on a medium prepared as follows:
(1) Infuse one part finely chopped choice lean beef in two parts distilled water in the ice box for 24 hours.
(2) Strain.
(3) Heat the juice to coagulate the proteins. Filter.
(4) Dissolve 1.0% Witte's peptone and 0.5% Kahlbaum's NaCl in the filtered infusion.
(5) Adjust to pH = 7.4.
(6) Boil and filter.
(7) Dissolve 2.0% agar in (6).
(8) Tube or distribute in flasks.
(9) Cool sterile melted tubes of (8) to 50°C. and add an equal volume of sterile defibrinated rabbit blood.
(10) Mix well and slant.
(11) Autoclave (8) at 110°C. for 20 minutes. Blood collected and defibrinated under aseptic conditions.

References: Smedley (1905 p. 28), Gurd (1908 p. 303), Duval (1910 p. 653), Hagemeister (1914 p. 228), Warden (1915 p. 428), Holman (1916 p. 381), Kohlman (1919 p. 574), Bernstein and Lowe (1919 p. 78), Wahl, White and Lyall (1919 p. 420), Yoshida (1920 p. 361), Bell (1920 p. 464), Anderson and Schultz (1921 p. 656), Harvey (1921–22 pp. 73, 74, 76), Jones (1922 p. 363), Pitfield (1922 p. 119), Stitt (1923 pp. 43, 44, 52), Klimmer (1923 p. 226), Park, Williams and Krumwiede (1924 p. 133), Soule (1925 p. 248).

1894. Harvey's Peptic Blood Digest Agar

Constituents:

1. Infusion agar	
2. NaCl (0.85% solution)	150.0 cc.
3. HCl	6.0 cc.

4. Blood, defibrinated sheep	50.0 cc.
5. Pepsin	1.0 g.
6. NaOH (20.0%)	12.0 cc.

Preparation:

(1) Prepare by addition in the order given: 0.85% sterile salt solution 150.0 cc.; hydrochloric acid 6.0 cc.; defibrinated sheep blood 50.0 cc., granulated pepsin B.P. 1.0 g.

(2) Shake to dissolve.

(3) Heat in the water bath at 55°C. for 2 to 24 hours with occasional shaking. (Note: The exact time is immaterial.)

(4) Add 12.0 cc. of 20.0% sodium hydroxide.

(5) Adjust the reaction by addition of 20.0% sodium hydroxide until a sample gives with cresol red indicator solution (0.02%) the color of permanganate (corresponding to pH = 7.6).

(6) Add pure Hydrochloric acid drop by drop until cresol red indicator solution gives practically no change of color but phenol red gives red. Note: Corresponding to pH = 7.0 to 7.2.

(7) Add chloroform to 0.25%.

(8) Shake to mix.

(9) Keep in a tightly stoppered bottle till required for use.

(10) When ready for use, mix at 45°C. 100 parts melted infusion agar (see variant (v) medium 1661) with 3.5 parts 9.

Sterilization: Not specified.

Use: Cultivation of *B. influenzae.*

Reference: Harvey (1921–22 p. 100).

1895. Bailey's Hormone Blood Agar

Add 5 parts per 100 of fresh defibrinated human blood to Bailey Hormone Agar (1673). This medium is recommended to preserve stock cultures of pneumococci and streptococci.

1896. Harvey's Ascitic Fluid Blood Agar

Constituents:

1. Infusion broth	30.0 cc.
2. Infusion agar	600.0 cc.
3. Blood, defibrinated, sheep	200.0 cc.
4. Ascitic fluid	100.0 cc.
5. Maltose	10.0 g.

Preparation:

(1) See variant (bb) medium 779 for preparation of infusion broth.

(2) See variant (v) medium 1661 for preparation of infusion agar.

(3) Mix 100 parts ascitic fluid, 200.0 cc. of defibrinated sheep blood, 10.0 g. maltose, dissolved in 30.0 cc. (1) with 600.0 cc. of (2).

Sterilization: Not specified.

Use: General culture medium.

Reference: Harvey (1921–22 p. 84).

1897. Harvey's Oxalated Blood Agar

Constituents:

1. Infusion agar	
2. Blood, ox or sheep	400.0 cc.
3. NaCl (0.85% solution)	
4. Ammonium oxalate (1.0% solution)	30.0 cc.

Preparation:

(1) Collect 400.0 cc. ox or sheep blood at the slaughter house in a sterile flask containing 30.0 cc. of a 1.0% solution of ammonium oxalate and 0.5 cc. formalin.

(2) Mix well.

(3) Allow to stand 30 minutes.

(4) Dilute ¼ with 0.85% sterile salt solution.

(5) Leave for 48 hours.

(6) Mix one part of oxlated blood with fifteen parts of infusion agar at 45°C. (see variant (v) medium 1661 for preparation).

Sterilization: Not specified.

Use: Cultivation of gonococci, meningococci and pneumococci.

Reference: Harvey (1921–22 p. 77).

1898. Wolbach, Chapman and Steven's Citrated Blood Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Veal	500.0 g.
3. Glucose	1.5 g.
4. NaCl	6.0 g.
5. Agar agar	14.0 g.
6. Blood, citrated rabbit	

Preparation:

(1) Prepare a veal infusion from 500.0 cc. of distilled water and 500.0 g. of lean veal. Exact method not given.

(2) Evaporate (1) to 100.0 cc.

(3) Dissolve 3, 4, and 5 in 900.0 cc. distilled water
(4) Add (2) to (3).
(5) Tube.
(6) Add 2.0 to 3.0 cc. of a mixture of equal parts of sterile rabbits blood and citrate saline solution to 4.0 cc. of the sterile agar jelly.
(7) Heat the mixture to 45°C. for 30 minutes.
(8) Slant, and allow to stand until a considerable water of condensation has collected, before inoculation.

Sterilization: Sterilize (5) method not given.

Use: Cultivation of trypanosomes. Authors reported that medium afforded rapid growth and the vitality of the cultures were not diminished. Gates cultivated meningococci on a similar medium.

Variants: Gates prepared a similar medium as follows:
(1) Prepare a veal infusion agar.
(2) Add 1.0% glucose to (1).
(3) Adjust to pH = 7.4.
(4) Sterilization not specified.
(5) Just before tubing add 5.0% sterile, unheated citrated horse plasma. (Fresh rabbit serum may be employed instead of plasma.)

References: Wolbach, Chapman and Stevens (1915–16 p. 109), Gates (1919 p. 322).

1899. Noguchi's Serum Plasma Agar

Constituents:

1. Ringer's solution	300.0 cc.
2. Serum rabbit	100.0 cc.
3. Plasma, citrated, rabbit	50.0 cc.
4. Agar (2.0%)	5.0 to 100.0 cc.

Preparation:
(1) Prepare 2.0% agar.
(2) Mix one part rabbit serum, three parts Ringer's solution (see medium 180), one-half part citrated rabbit plasma and one-half to one part neutral or slightly alkaline (1). The agar is to be melted and cooled to 60 to 65°C. before mixing with the other materials.

Sterilization: Not specified.

Use: Isolation of *Spirochaeta icterohaemrrhagiae*. Inoculate with suspected blood (material) and cover with a thin layer of sterile paraffin oil. Author reported that growth took place at any temperature from 10°C. to 37°C. Growth was nearly invisible and about 1.0 to 1.5 cm. below the surface.

Reference: Noguchi (1917 p. 761).

1900. Sparkar's Citrated Blood Agar (Liston)

Constituents:

1. Water	2000.0 cc.
2. Meat	1000.0 cc.
3. Peptone	40.0 g.
4. NaCl	20.0 g.
5. Agar	60.0 g.
6. Blood (human)	

Preparation:
(1) Boil 1000.0 g. of meat in 2 liters of water for 40 minutes.
(2) Filter.
(3) Dissolve 3, 4, and 5 in sterile (2).
(4) Draw one volume of human blood into a flask containing 4 volumes sterile citrated saline (0.5% sodium citrate and 0.85% NaCl).
(5) Mix the blood and saline thoroly.
(6) Heat in a water bath at 65°C. for 30 minutes.
(7) Remove precipitate by filtering thru sterilized filter paper.
(8) Add 1.0 cc. of (7) to 10.0 cc. of melted (3) at 50°C.
(9) Slant.

Sterilization: Sterilize (2) at 15 pounds pressure in the autoclave

Use: Cultivation of *B. influenzae* (Pfeiffer).

Variants:
(a) Malone prepared a similar medium as follows:
  (1) Method of preparation or exact composition of nutrient agar not given.
  (2) Add 10.0 cc. of pigeons blood to 90.0 cc. of 1.0% sodium citrate solution in normal salt solution.
  (3) Heat (2) for ½ hour at 66° to 70°C.
  (4) Filter thru filter paper.
  (5) Add 1.0 cc. of (4) to 10.0 cc. of nutrient agar at a temperature of 50° to 60°C.

- (6) Method of sterilization not given.
- (7) Medium is a faint yellow in color and transparent.

(b) Harvey prepared a medium as follows:

- (1) Add 10.0 cc. of blood to 1.0 cc. sterile 10.0% sodium citrate.
- (2) Add 1.0 cc. of (1) to a test tube of melted infusion agar at 45°C. (see variant (v) medium 1661 for preparation).
- (3) Rotate the test tubes between the hands to distribute the blood thru the agar.

(c) Harvey gave the following method of preparation:

- (1) Add 10.0 cc. of pigeon's blood to 90.0 cc. of a 1.0% citrated 0.85% sterile NaCl solution.
- (2) Heat 30 minutes at 65 to 70°C.
- (3) Filter thru thick filter paper.
- (4) Mix 1.0 cc. of the filtrate from (3) with 10.0 cc. of melted infusion, cooled to 45°C. with a reaction slightly alkaline to litmus. (See variant (v) medium 1661 for preparation of agar.)

(d) Soparkar (Harvey) prepared a similar medium as follows:

- (1) Mix 10.0 cc. of fresh human blood with 40.0 cc. of a 0.5% citrated 0.85% NaCl solution.
- (2) Heat in a water bath 30 minutes at 64 to 68°C.
- (3) Filter thru sterilized paper.
- (4) Mix 1.0 cc. of the filtrate from (3) with 10.0 cc. of melted infusion, cooled to 45°C. with a reaction slightly alkaline to litmus. (See variant (v) medium 1661 for preparation of agar.)

References: Liston (1918–19 p. 418), Malone (1919–20 p. 504), Harvey (1921–22 p. 77).

1901. Hirsch and McKinney's Chocolate Agar

Constituents:

1. Infusion agar	1000.0 cc.
2. Blood (citrated horse 1.0 to 2.0%)	10.0 to 20.0 cc.

Preparation:

- (1) Prepare beef infusion agar.
- (2) Adjust to a reaction of 0.2%.
- (3) Draw horse blood into flasks containing sterile sodium citrate solution to make the final concentration 1.5%.
- (4) After corpuscles have settled, the plasma is removed and sterile distilled water added to make up the original volume of the blood.
- (5) Heat sterile (2) to 90°C. and add sterile laked blood up to 1.0 to 2.0%.

Sterilization: Method not specified.

Use: Isolate influenza bacilli.

Variants: Harvey prepared a similar medium as follows:

- (1) Mix 5 parts infusion agar at 45°C. (see variant (v) medium 1661 for preparation) with 2/10ths part of citrated blood at 45°C. (one part 10.0% citrate in 0.95% sterile salt solution to ten parts blood).
- (2) Place in a water bath at 80°C. until the agar has become chocolate in color.
- (3) Slope.

References: Hirsch and McKinney (1919 p. 605), Harvey (1921–22 p. 77).

1902. Harvey's Glucose Blood Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Ox heart	1000.0 g.
3. Peptone	10.0 g.
4. Na_2HPO_4	10.0 g.
5. Agar	30.0 g.
6. Glucose	25.0 g.
7. Citrated blood	

Preparation:

- (1) Mix 1000.0 cc. distilled water with 1000.0 g. ox heart.
- (2) Heat the mixture 20 minutes at a temperature not exceeding 50°C., with constant stirring.
- (3) Raise the temperature to boiling point.
- (4) Boil 10 minutes.
- (5) Pour the mixture on to a wet, thick, clean cloth.
- (6) Collect the fluid which drains thru the cloth together with that obtained by squeezing the meat in the cloth.
- (7) Add peptone 1.0%; di-sodium phosphate 1.0%, prepared agar (see medium 1401) 3.0%.
- (8) Steam gently 2½ hours to dissolve the agar.

(9) Allow to cool to 60°C.
(10) Clarify with white of egg.
(11) Bring the volume to its orginal amount.
(12) Estimate and make the reaction 0.6% acid to phenolphthalein.
(13) Add to the melted nutrient agar, 2.5% glucose.
(14) Steam 30 minutes. (Note: It is better to rely on one steaming for sterilization than to sterilize by steaming on three successive days.)
(15) Distribute in quantities of 4.0 cc. into test tubes.
(16) Keep until required for use in the ice chest.
(17) Prepare sterile 10.0 cc. centrifuge tubes each containing 2.0 cc. sterile 2.0% sodium citrate.
(18) Have in readiness corks or rubber bungs, contained in alcohol to fit the centrifuge tubes.
(19) Fill up the centrifuge tubes with human blood sterilely aspirated.
(20) Replace the wool plugs of the centrifuge tubes by corks after burning off the alcohol.
(21) Centrifuge.
(22) Prepare with sterile precautions: Centrifuged blood fluid at 45°C. (21) 75; melted nutrient agar (13) contained in the test tubes at 45°C. 4.
(23) Roll the test tubes between the hands to mix.
(24) Test sterility by incubating 48 hours.

Sterilization: See step (14) under preparation.

Use: Cultivation of gonococci.

Reference: Harvey (1921–22 p. 82).

1903. Grassberger's Blood Agar

Constituents:
1. Infusion agar.
2. Blood.

Preparation:
(1) Prepare beef infusion agar.
(2) Add 10.0% NaOH until blue litmus paper is no longer blue.
(3) Distribute in 20.0 cc. lots.
(4) Add 20 drops diluted 10.0% soda solution to each flask so as to obtain a normal agar (10.0 cc. N/1 soda solution, for 1000.0 cc. agar). (The exact amount of soda solution, or dilution of soda solution was not specified.)
(5) Pour into plates.
(6) Smear 1.0 cc. of blood over each plate.

Sterilization: Not specified.

Use: Cultivation of influenza bacilli. Other investigators employed similar media for various purposes.

Variants:
(a) Waksman studied the metabolism of actinomycetes on a medium prepared as follows:
  (1) Boil 500.0 g. veal with 1000.0 cc. tap water for 10 minutes.
  (2) Filter.
  (3) Add 10.0 g. Bacto peptone, 5.0 g. NaCl, and 25.0 g. agar and dissolve. (Heat).
  (4) Adjust to +1.0 (pH = 7.6–7.8).
  (5) Flask and sterilize.
  (6) Cool to 45°–50°C. and add 10.0% (100.0 cc.) sterile whole rabbit blood. Shake.
  (7) Pour, under sterile conditions, into sterile test tubes or Petri dishes.
  (8) Slant tubes.
(b) Twort and Twort prepared a similar medium as follows:
  (1) Prepare infusion agar and infusion broth.
  (2) Sterilize the broth in a flask.
  (3) Add 5.0% rabbit blood, withdrawn from the vein of the ear with a sterile syringe, to (2).
  (4) Incubate (3) at 37° for one hour, shaking repeatedly to prevent the forming of too much clot.
  (5) Melt infusion agar tubes and cool to 50°C.
  (6) Add about 20 drops of the fluid from (4) to each tube of (5).
  (7) Slant.
(c) Harvey gave the following method of preparation:
  (1) Wash and scrub the finger to be used well with hot soap and water.
  (2) Drop on to the finger absolute alcohol followed by ether to remove the alcohol.
  (3) Congest the pulp of the finger by winding a bandage round the base.
  (4) Prick the congested pulp with a sterile needle.

(5) Take up a drop of the blood which exudes in a sterile platinum loop.
(6) Smear on the surface of an agar slope. (Serum may be used in the same way.)
(7) Cover the test tube with an India-rubber cap.
(8) Test sterility before use by incubation 48 hours.

(d) Harvey also prepared a medium as follows:
  (1) Add 0.25 cc. blood to 5.0 cc. melted agar infusion in a test tube. (See variant (v) medium 1661 for preparation.)
  (2) Boil one minute.
  (3) Slope. (The precipitate settles to the bottom.)

(e) Harvey prepared a similar medium as follows:
  (1) Add directly, blood drawn off from a vein or from the heart with a sterile syringe to melted infusion agar in a test tube at 45°C. (See variant (v) medium 1661.)
  (2) Roll the test tube between the palms to mix.
  (3) Slope.
  (4) Test sterility by incubation 48 hours.

References: Grassberger (1897 p. 462), Waksman (1919 p. 210), Twort and Twort (1921 p. 88), Harvey (1921–22 p. 72).

1904. Ruediger's Blood Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Veal, minced	500.0 g.
3. Peptone, Bacto	10.0 g.
4. Bacto agar	15.0 g.
5. NaCl	5.0 g.
6. Blood, human	100.0 cc.

Preparation:

(1) Boil 2 in 1 for one hour.
(2) Cool and strain thru cheese cloth.
(3) Neutralize to phenolphthalein with N/1 NaOH.
(4) Dissolve 3, 4 and 5 in (3).
(5) Tube in 10.0 cc. lots.
(6) Heat human blood to 56°C. for 30 minutes.
(7) Add 1.0 cc. of (6) to 10.0 cc. of sterile (5).
(8) Slant and solidify.
(9) Stopper tubes air tight with sterile cork stoppers.

Sterilization: Sterilize in the autoclave.

Use: Cultivation of gonococci. The author reported that the addition of 1.0% glycerol or 1.0% glucose inhibited growth just as did the absence of peptone.

Reference: Ruediger (1919 p. 377).

1905. Dieudonne's Alkaline Blood Agar (Tanner)

Constituents:

1. Infusion agar	700.0 cc.
2. Blood, beef	150.0 cc.
3. KOH (N/1)	150.0 cc.

Preparation:

(1) Mix equal volumes of beef blood and normal KOH solution.
(2) Steam for 30 minutes.
(3) Mix 3 volumes of (2) with 7 volumes of neutral (to litmus) infusion agar.
(4) Pour into plates.
(5) Dry the plates at 60°C. for 30 minutes, and at room temperatures for 24 hours before use.

Sterilization: Not specified.

Use: Cultivation of *Microspira cholerae.*

Reference: Tanner (1919 p. 70).

1906. Sherwood and Downs' Glucose Blood Agar

Constituents:

1. Infusion agar	1000.0 cc.
2. Glucose	20.0 g.
3. Blood (whole)	50.0 cc.

Preparation:

(1) Prepare meat infusion agar with a reaction of +0.2% to phenolphthalein.
(2) Add 20.0 g. glucose and 50.0 cc. of whole blood to 1000.0 cc. of (1).

Sterilization: Not specified.

Use: To determine hemolytic ability of streptococci.

Reference: Sherwood and Downs (1919 p. 135).

1907. Hall's Testicular Infusion Blood Agar (Stitt)

Constituents:

1. Distilled water	1000.0 cc.
2. Testicles, beef	500.0 g.
3. Peptone (2.0%)	20.0 g.
4. Glucose (0.5%)	5.0 g.
5. NaH_2PO_4 (0.2 to 0.3%)	2.0 to 3.0 g.

6. Agar (2.5%)	25.0 g.
7. Blood, human (0.5 to 2.5%)	5.0 to 25.0 cc.

Preparation:

(1) Remove all connective tissue from beef testicles and put thru a meat grinder.
(2) Cover (1) with twice its weight of distilled water.
(3) Infuse on ice over night.
(4) Heat to 50°C. in a double boiler on the following morning.
(5) Allow to stand for an hour and then raise to the boiling point.
(6) Allow to stand for another hour to permit the settling of the solid particles.
(7) Decant the liquor.
(8) Add 2.0% peptone, 0.5% glucose, 0.2 to 0.3% NaH_2PO_4 and 2.5% granular agar.
(9) Heat over a free flame until solution is complete, stirring constantly.
(10) Adjust the reaction to $pH = 7.4$ to 7.8, using phenol red as an indicator.
(11) Tube.
(12) Check the reaction after sterilization.
(13) Add human blood in the proportion of 0.5 to 2.5% to the tubes while they are still liquid (just before the agar solidifies).

Sterilization: Sterilize (1) by heating at 15 pounds pressure for 15 minutes.

Use: Cultivation of gonococci.

Variants: The author gave the following variants:

(a) Defibrinated rabbits blood (1.0 to 5.0%) may be added instead of human blood.
(b) The blood may be added before sterilization. This gives a "chocolate" colored medium.

Reference: Stitt (1923 p. 46).

1908. Cutler's Blood Clot Infusion Agar (Klimmer)

Constituents:

1. Water	1000.0 cc.
2. Blood clot	500.0 g.
3. Peptone (1.0%)	10.0 g.
4. NaCl (0.5%)	5.0 g.
5. Blood	

Preparation:

(1) Boil 500.0 g. human blood clot with a liter of water.
(2) Filter.
(3) Add 0.5% NaCl and 1.0% peptone to the filtrate.
(4) Distribute as desired (tube).
(5) Add several drops of blood to each tube of sterile (4) when ready for use.

Sterilization: Method of sterilization of (4) not given.

Use: Cultivation of amoeba.

Variants: Klimmer infused placenta instead of blood clot.

Reference: Klimmer (1923 p. 229).

1909. Harvey's Trypsinized Blood Agar

Constituents:

1. Infusion agar.
2. Trypsin.
3. Blood, human.

Preparation:

(1) Add 0.25 g. of anhydrous Na_2CO_3 to 100.0 cc. of a 5.0% trypsinized 0.85% sterile salt solution under aseptic conditions.
(2) Distribute in quantities of 10.0 cc. in sterile test tubes.
(3) Add to each test tube 2.0 cc. sterile human blood.
(4) Incubate 8 days or longer with occasional shaking.
(5) Add one part of (4) at 45°C. to nine parts of melted infusion agar at 45°C. (see variant (v) medium 1661 for preparation).
(6) Distribute into test tubes or plates.

Sterilization: Method not given.

Use: Cultivation of *B. influenzae*.

Reference: Harvey (1921–22 p. 77).

1910. Harvey's Hydrolyzed Blood Agar

Constituents:

1. Infusion agar	350.0 cc.
2. Water	50.0 cc.
3. Blood	10.0 cc.
4. H_2SO_4 (normal)	10.0 cc.

Preparation:

(1) Mix one part of blood, one part normal H_2SO_4 with 5 parts water.
(2) Boil.
(3) Store for use.
(4) Make faintly alkaline to litmus at the time of use.

(5) Mix one part of (4) at 45°C. with five parts melted infusion agar at 45°C. (See variant (v) medium 1661 for preparation.)

Sterilization: Not specified.

Use: Isolation of *B. influenzae.*

Variants: Author reported that Brilliant green which inhibits the gram + bacteria may be added.

Reference: Harvey (1921–22 p. 76).

1911. Kristensen's Hemoglobin Infusion Agar

Constituents:

1. Water	25,000.0 cc.
2. Meat	25,000.0 g.
3. Peptone	1.0%
4. NaCl	0.5%
5. Hemoglobin	1.0%

Preparation:

(1) Mince 25,000.0 g. beef.
(2) Add 25 liters of water and allow to stand in the cold until the next day.
(3) Boil for a short time.
(4) Press the fluid from the meat.
(5) Add about 16 liters of water to the meat, heat to boiling once more and press the juice again from the meat.
(6) Mix the fluid from (4) and (5).
(7) Add 1.0% peptone and 0.5% NaCl.
(8) Adjust to pH of about 7.8 to 7.9.
(9) Dissolve 2.0% agar in (8). (Agar may be omitted giving a liquid medium.)
(10) Filter thru cotton wool and distribute in 600.0 cc. flasks.
(11) The pH of the sterile agar should be about 7.2.
(12) Allow a flask containing defibrinated blood to stand several hours to permit the corpuscles to sink.
(13) Pour off the supernatant fluid and replace with distilled water.
(14) Distribute the hemoglobin solution thus obtained in 20.0 cc. quantities.
(15) Store in the ice box until ready for use.
(16) Add 20.0 cc. of (15) to 600.0 cc. of sterile melted (11) cooled to 40 to 50°C.
(17) Pour into plates.

Sterilization: Sterilize (10) in the steamer on each of 3 successive days.

Use: Cultivation of hemoglobinophilic bacteria.

Reference: Kristensen (1922 p. 231).

1912. Brown and Orcutt's Hemoglobin Infusion Agar

Constituents:

1. Veal infusion agar.
2. Hemoglobin.

Preparation:

(1) Prepare veal infusion agar.
(2) Defibrinate blood and wash repeatedly with sterile physiological salt solution.
(3) Lake the washed corpuscles with distilled water and remove the corpuscles stroma by centrifugation. (Centrifuge the laked blood corpuscles until the supernatant hemoglobin solution no longer gives a clouding reaction with salt.)
(4) Add the hemoglobin solution thus obtained to melted agar (amounts not specified) and pour in plates.

Sterilization: Not specified.

Use: To determine food requirements for *Bacillus pyogenes.*

Reference: Brown and Orcutt (1920 p. 223).

1913. Esch's Alkaline Hemoglobin Infusion Agar (Tanner)

Constituents:

1. Infusion agar	85.0 cc.
2. Hemoglobin (horse) (Merck)	5.0 g.
3. KOH (N/2)	30.0 cc.

Preparation:

(1) Dissolve 5.0 g. of Merck's horse hemoglobin in 30.0 cc. of half normal KOH.
(2) Steam for 30 minutes.
(3) Mix 15.0 cc. of (2) with 85.0 cc. of neutral (litmus) infusion agar.
(4) Pour in plates.
(5) Dry at room temperatures with covers removed.

Sterilization: Not specified.

Use: Cultivation of *Microspira cholerae.*

Reference: Tanner (1919 p. 70).

1914. Sherwood and Downs' Basal Serum Agar

Constituents:

1. Infusion agar	1000.0 cc.
2. Serum	20.0 cc.
3. Andrade's Indicator	

Preparation:

(1) Prepare meat infusion agar and adjust to a reaction of +0.2% to phenolphthalein.
(2) Dilute serum with distilled water 1:3.
(3) Add 5.0 cc. of (2) to each 100.0 cc. of (1).
(4) Add under aseptic conditions a sufficient amount of a sterile solution of one of the added nutrients so that it be present in 2.0% strength in sterile (3).
(5) Add Andrade's indicator.
(6) Distribute directly into sterile test tubes in 8.0 cc. lots and slant and inoculate as in Russell's medium.
(7) Incubate to determine contamination.

Sterilization: Sterilize (3) in the autoclave. Method of sterilization of solutions of added nutrients not given.

Use: To determine fermentation of carbohydrates by streptococci. Authors reported that this medium supported the growth of pneumococci and quite a number of the delicate growing streptococci.

Added nutrients: The authors added 2.0% of any desired carbohydrate.

Reference: Sherwood and Downs (1919 p. 135).

1915. Harvey's Placenta Blood Serum Agar

Constituents:

1. Infusion agar.
2. Serum, Placental blood.

Preparation: (1) Mix equal parts of sterile or sterilized placental blood at 45°C. with infusion agar at 45°C., 1.0% acid to phenolphthalein. (See variant (v) medium 1661 for preparation.)

Sterilization: Not specified.

Use: General culture medium.

Reference: Harvey (1921–22 p. 78).

1916. Veillon's Serum Agar

Constituents:

1. Infusion agar	100.0 cc.
2. Serum (human)	100.0 cc.

Preparation:

(1) Prepare infusion agar and cool to 40°C.
(2) Heat serum at 38 to 40°C.
(3) Mix equal parts of (1) and (2).

Sterilization: Not specified.

Use: Cultivation of gonococci. Other investigators used similar media for the cultivation of a large variety of organisms.

Variants: The following authors prepared media as indicated below:

(a) Joos used the following medium for the diagnosis of diphtheria. He reported that diphtheria colonies may appear after 4–5 hours as small grey white colonies, of moist appearance which can easily be recognized. Streptococci and staphylococci were inhibited. Serum need not be collected under aseptic conditions.
  (1) Extract 500.0 g. beef (several days old) with 1000.0 cc. water.
  (2) Filter and dissolve 20.0 g. peptone and 5.0 g. NaCl in (1).
  (3) Make alkaline with N/1 NaOH by adding (about 7.0 to 8.0 cc. of normal solution per liter) to neutral medium.
  (4) To 300.0 cc. of blood serum add 50.0 cc. of N/1 NaOH solution and 150.0 cc. of (3). (Distilled water may be used instead of (3).
  (5) Place the mixture in a flat bottomed flask and place the flask in a water bath at about 60° to 70°C. for 2 to 3 hours.
  (6) Raise the temperature to 100°C., or better, place in a steamer for 30 to 45 minutes.
  (7) Then add an equal amount (500.0 cc.) of (3) and 20.0 g. of agar agar.
  (8) Dissolve the agar as quickly as possible.
  (9) When solution is complete, filter in hot condition and sterilize for 15 minutes at 100 to 110°C. in an autoclave.
  (10) Pour into sterile Petri dishes.

(b) Behrens cultivated *Trypanosoma Brucei* on the following medium:
  (1) Digest chopped beef in 250.0 cc. water over night in the cold, or for one hour at 55°C.
  (2) Strain (1), boil the extract and filter.
  (3) Dialyze the filtrate in a large collodium sac against running distilled water for 24 to 48 hours.

(4) Dilute (3) to 1000.0 cc.
(5) Dissolve 20.0 g. peptone, 5.0 g. NaCl, 0.1 g. $CaCl_2$, 10.0 cc. N/1 Na_2CO_3 solution, and 20.0 g. agar in (4).
(6) Distribute in test tubes in 1.0 cc. lots.
(7) Sterilize in autoclave by heating to 105–108° for 15 minutes.
(8) Centrifuge defibrinated rabbit blood and draw off serum.
(9) Dilute serum with 0.5% NaCl solution to original blood volume.
(10) Shortly before use melt the desired tubes of agar, cool to 60°C. and add two volumes of (9) to each tube.
(11) Mix well and solidify in slanting positions.

(c) Ferry and Noble cultivated *Diplococcus pneumoniae*, *Streptococcus viridans* and *Streptococcus hemolyticus* on a medium prepared as
(1) Add 500.0 g. of finely chopped lean veal to 1000.0 cc. of water.
(2) Macerate and allow to stand over night.
(3) Strain thru cheese cloth and bring to a boil.
(4) Filter.
(5) Add 20.0 g. peptone (this may be omitted) 5.0 g. NaCl and 30.0 g. of finely chopped agar.
(6) Boil and adjust the reaction to the neutral point, using phenolphthalein as an indicator.
(7) Filter.
(8) Tube in 3.0 cc. quantities and sterilize the fractional method in the steamer.
(9) When ready for use, melt the agar, cool to 45°C. and add 2.0 cc. of sterile normal horse serum to each tube.

(d) Ball.
(1) Add 500.0 g. lean chopped veal to 1000.0 cc. water, 20.0 g. peptone, 5.0 g. NaCl and 30.0 g. finely chopped agar.
(2) Adjust reaction.
(3) Sterilize by fractional method.
(4) Cool to 45°C.
(5) Add 2.0 cc. of sterile normal horse serum.

(e) Harvey.
(1) Mix two parts melted infusion agar at 45°C. (see variant (v) medium 1661) with one part sterile human serum at 45°C.

(f) Harvey cultivated meningococci on a medium prepared as follows:
(1) Collect ox or sheep blood at the slaughter house in a sterile blood jar.
(2) Leave the jar at the slaughter house to avoid the shaking up consequent on transportation.
(3) Transfer the separated serum 24 hours later with a sterile pipette to a sterile flask.
(4) Transport to laboratory.
(5) Add ether to 0.5%.
(6) Cork tightly.
(7) Leave 24 hours.
(8) Replace the cork with a sterile wool plug.
(9) Keep the flask and contents in a water bath for 3 hours at 50°C. to drive off the ether.
(10) Mix 25 parts (9) at 45°C. with 100 parts melted infusion agar (see variant (v) medium 1661).

(g) Jones cultivated an organism resembling *Bacillus actinoides* from pneumonic rat lungs on a medium prepared as follows:
(1) Prepare veal infusion agar.
(2) Slant.
(3) Add 0.5 cc. of calf serum water (or defibrinated horse blood) to the water of condensation.
(4) Remove small pieces of lung tissue from an infected rat under aseptic conditions.
(5) Push the pieces of tissue down in the tube into the liquid.
(6) Seal the tubes with sealing wax.

(h) Fitch cultivated *Bact. abortus* Bang on a medium prepared as follows:
(1) Prepare beef infusion broth.
(2) Dissolve 20.0 g. agar in (1).
(3) Adjust to pH from 6.8 to 7.2.
(4) Tube.
(5) Method of sterilization not given.
(6) At time of use melt agar and add (10.0%) naturally sterile horse serum.

  - (7) Cool and solidify in slanting position.
  - (8) Inoculate tubes and place in a 10.0% atmosphere of CO_2.
- (i) Kinsella, Brown and Garcia used the following medium for the isolation of gonococci:
  - (1) Prepare 1.6% beef or veal infusion agar.
  - (2) Adjust to pH = 7.6.
  - (3) Distribute into 100.0 cc. quantities in Erlenmeyer flasks and sterilize (method not given).
  - (4) When ready for use melt (3) and add to each flask containing 100.0 cc. of hot agar (90–100°C.) 30.0 cc. of (sterile) beef serum.
  - (5) Mix thoroly, distributing the small particles of coagulated serum thru the medium.
  - (6) Pour into sterile Petri dishes, cover with porous terracotta lids and place in incubator over night (18 hours).
  - (7) Replace terra-cotta lids by glass lids and the medium is read for inoculation.

  Authors reported that gonococci reached maximum growth in 48 hours. The type of serum used had little or no influence on the value of the medium. Plates should never dry under the terra-cotta lids longer than 24 hours.

References: Veillon (1898 p. 22), Joos (1899 p. 303), Behrens (1914 p. 29), Ferry and Davis (1918 p. 295), Ball (1919 p. 82), Harvey (1921–22 pp. 79, 80, 82), Jones (1922 p. 363), Fitch (1922 p. 234), Kinsella, Brown and Garcia (1923 p. 1).

1917. Kutscher's Serum Placenta Agar

Constituents:

1. Water	1000.0 cc.
2. Placenta	500.0 g.
3. Agar	25.0 g.
4. NaCl	5.0 g.
5. Glucose	10.0 g.
6. Nutrose	20.0 g.
7. Peptone (Chapoteaut)	20.0 g.
8. Serum (Beef)	335.0 cc.

Preparation:

- (1) Cut fresh placenta into small pieces.
- (2) Weigh the pieces and juice and add a double weight of water.
- (3) Prepare 2.5% agar in the usual way, adding 0.5% NaCl, 1.0% glucose, 2.0% nutrose and 2.0% Chapoteaut's peptone. The reaction is slightly alkaline.
- (4) Distribute in 100.0 cc. lots.
- (5) To 3 parts sterile (4) add 1 part sterile beef serum.
- (6) Mix well and pour into plates. The reaction is slightly alkaline.

Sterilization: Method of sterilization of (4) not given. Sterilize beef serum in 50.0 cc. lots by placing it at 60°C. for one hour on 4 successive days.

Use: Cultivation of meningococci. Author reported that after 18–24 hours at 37°C. meningococci colonies were circular, light transparent, grayish-blue and about 2–3 mm. in diameter.

Variants:

- (a) Scott gave the following method of preparation:
  - (1) Mince the placenta and prepare a boiled extract as with meat (exact method not given).
  - (2) Add 2.0% nutrose, 1.0% glucose, 1.5% peptone (Chapoteaut) and 2.5% agar. Dissolve by steaming.
  - (3) Adjust the reaction to a +8 (Eyre).
  - (4) Flask in 75.0 cc. lots.
  - (5) Sterilize (method not given).
  - (6) When ready for use melt and cool to 50°C.
  - (7) Add 25.0 cc. of sterile (filtered) ox serum to each flask.
  - (8) Mix and pour at once into plates.
- (b) Klimmer specified the use of 2.0% agar instead of 2.5%.

References: Kutscher (1908 p. 287), Scott (1915–17 p. 466), Harvey (1921–22 p. 79), Klimmer (1923 pp. 202, 225).

1918. Bezançon's Serum Placenta Agar

Constituents:

1. Water	1000.0 cc.
2. Placenta	500.0 g.
3. Agar	25.0 g.
4. Glucose	10.0 g.
5. Peptone	20.0 g.
6. NaCl	5.0 g.
7. Serum	300.0 cc.

Preparation:

- (1) Soak 500.0 g. of finely chopped placenta in 1000.0 cc. of water in the ice box over night.

(2) Filter.
(3) Dissolve 2.5 g. agar, 0.5 g. NaCl, 1.0 g. glucose and 2.0 g. peptone in each 100.0 cc. of the filtrate.
(4) Heat beef serum at 60°C. for one hour on each of 4 successive days.
(5) To three parts (3) add one part (4).
(6) Mix well.
(7) Distribute in sterile containers.

Sterilization: Sterilization of (3) not specified.

Use: General culture medium.

Reference: Bezançon (1920 p. 119).

1919. Shmamine's Nucleic Acid Serum Agar

Constituents:

1. 3.0% agar with 0.5% glucose	100.0 cc.
2. Serum	100.0 cc.
3. Physiological salt solution	10.0 cc.
4. Sodium salt of nucleic acid	0.5 to 1.0 g.

Preparation:

(1) Dissolve 0.5 to 1.0 g. of the sodium salt of nucleic acid in 10.0 cc. physiological salt solution.
(2) Add sterile (1) to 100.0 cc. sterile clear transparent serum (or just 10.0 cc. of sterile physiological salt solution may be added).
(3) Distribute into sterile test tubes.
(4) Prepare a 3.0% infusion agar containing 0.5% glucose.
(5) Melt (4) and cool to 50°C.
(6) Mix equal parts of sterile (5) and sterile (3).

Sterilization: Sterilize (1) for 15 minutes in boiling water bath. Sterilize (3) by heating for one hour on each of 3 successive days at 60°C. Method of sterilization of (5) not given.

Use: Isolation of spirochetes.

Reference: Shmamine (1912 p. 317).

1920. Robey, et al., Glucose Serum Agar

Constituents:

1. Veal infusion agar	1000.0 cc.
2. Glucose	5.0 g.
3. Serum, horse	50.0 g.

Preparation:

(1) Prepare veal infusion agar.
(2) Adjust reaction to 0.3 to 0.5% acid to phenolphthalein.
(3) Add 2 and 3 to (2).

Sterilization: Not specified.

Use: Isolation of meningococci. Authors reported that after 24 hours meningococci produced a colony 0.5–1.5 mm. in diameter, easily recognizable from influenza bacilli, streptococci and others.

Reference: Robey, Saylor, Meleney, Ray and Landmann (1918 p. 324).

1921. Noguchi's Ringer Solution Serum Agar

Constituents:

1. Ringer solution	45.0 cc.
2. Rabbit serum	15.0 cc.
3. Agar 2.0%	10.0 cc.

Preparation:

(1) Prepare 2.0% agar.
(2) Mix 4.5 parts Ringer's (see medium 180) solution and 1.5 parts rabbit serum with 1.0 part 2.0% agar.

Sterilization: Not specified.

Use: Cultivation of *Leptospira icterohaemorrhagiae*. Medium was covered with a sterile layer of paraffin oil after inoculation.

Variants:

(a) Noguchi prepared a similar medium as follows:
  (1) Prepare a 2.0% agar.
  (2) Mix 1.5 parts rabbit serum, 4.5 parts Ringer's solution and 1.0 part 2.0% agar.
  (3) Allow (2) to solidify.
  (4) Add to solidified (3) a mixture of 1.5 parts rabbit serum and 4.5 parts Ringer's solution.
  (5) Cover with a layer of sterile paraffin oil.
(b) Harvey cultivated *S. icterohaemorrhagiae* on a medium prepared as follows:
  (1) Dissolve 0.88 g. NaCl, 0.025 g. KCl, 0.02 g. $CaCl_2$ and 0.015 g. Na_2CO_3 in 100.0 cc. distilled water.
  (2) Mix 1.5 parts of sterile rabbit serum at 45°C., 4.5 parts (1) at 45°C. and 1 part of melted infusion agar at 45°C. (See variant (v) medium 1661.)
  (3) Cover with a thin layer of paraffin oil.

References: Noguchi (1918 p. 606), Harvey (1921–22 p. 80).

1922. Todd's Lactose Serum Agar

Constituents:

1. Sugar free agar	1000.0 cc.
2. Lactose	20.0 g.
3. Neutral red (1.0% watery solution basic stain)	0.4 cc.
4. Serum, beef	200.0 cc.

Preparation:
(1) Prepare sugar free agar.
(2) Dissolve 2 and 3 in 1. (Dye strength such that medium is colored.)
(3) Tube in 7.0 to 8.0 cc. lots.
(4) Just before use add 2.0 cc. of sterile beef serum to each tube of sterile (3).

Sterilization: Sterilize for three successive days in Arnold. (Time not specified.)

Use: Isolation and differentiation of streptococci. Author reported that *Strept. pyogenes* formed deep red colonies with pink border. *Strept. viridans* (Schottmüller) formed red colonies surrounded by large area of milky-like haze; *Strept. mucosus* (Schottmüller) pink centered colonies with white border, edge irregular with milky-like haze. Pneumococci formed red centered colonies with narrow white border, smooth edge sharply defined. Other sugars and dyes were tried but this combination gave best results.

Reference: Todd (1910 p. 74).

1923. Harvey's Telluric Acid Serum Agar

Constituents:

1. Infusion agar	
2. Serum (sheep)	50.0 cc.
3. Telluric acid (1.0% solution)	9.0 cc.

Preparation: (1) Mix 50 parts sterilized sheep serum at 45°C., 9 parts of a sterile 1.0% telluric acid solution at 45°C., and 1000 parts of melted infusion agar, cooled to 45°C., neutral red to litmus (see variant (v) medium 1661 for preparation).

Sterilization: Not specified.

Use: Enrichment medium for *B. diphtheriae.*

Reference: Harvey (1921–22 p. 92).

1924. Francis' Cystine Serum Agar (Stitt)

Constituents:

1. Beef infusion	1000.0 cc.
2. Peptone (1.0%)	10.0 g.
3. Agar (1.0 or 1.5%)	10.0 or 15.0 g.
4. NaCl (0.5%)	5.0 g.
5. Cystine (0.1%)	1.0 g.
6. Glucose (1.0%)	10.0 g.
7. Serum horse (5.0%)	50.0 cc.

Preparation:
(1) Prepare beef infusion.
(2) Dissolve 2, 3 and 4 in (1).
(3) Adjust to pH = 7.3.
(4) Keep this as a stock agar.
(5) Add 0.1% cystine (or cystein hydrochloride) and 1.0% glucose to (4) when ready for use.
(6) Place in the Arnold for 15 minutes in flowing steam to melt the agar, and sterilize the cystine.
(7) Cool to 45 to 50°C. and add 5.0% sterile horse serum.
(8) Tube in sterile tubes.
(9) Slant.
(10) Incubate for 24 hours to test sterility.

Sterilization: Method of sterilization of (3) not given. Cystine sterilized in step (6) above.

Use: Cultivation of *Bacterium tularense,* gonococci and diphtheria bacilli.

Reference: Stitt (1923 p. 46).

1925. Elser and Huntoon's Basal Ascitic Fluid Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Lean beef	500.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Agar agar	25.0 g.
6. Litmus solution (Kubel & Tiemann)	15.0 cc.
7. Ascitic fluid	335.0 cc.

Preparation:
(1) Prepare basic litmus infusion agar from 1, 2, 3, 4, 5, 6 and one of the added nutrients in the same manner exactly as given in medium 1677. Use 25.0 g. agar instead of 16.0 g. agar.
(2) To the finished product which is cooled to 55°C. add ⅓ its volume of sterile ascitic fluid. The degree of alkalinity of the ascitic fluid must be previously determined and taken into consideration when correcting the reaction of the agar.

Sterilization: Not specified.

Use: Cultivation of meningococci, pseudomeningococci and gonococci. Authors reported that the medium was especially suited to freshly isolated cultures.

Added nutrients: The author added 1.0% of one of the following:

glucose
galactose
levulose
lactose
maltose
sucrose
mannitol
inulin
dextrin
dulcitol

Variants:

(a) Plotz, Olitsky and Baehr used the following medium to study fermentation by organisms causing typhus exanthematicus:
  (1) Prepare 3.0% agar.
  (2) Add 2.0% of one of the added nutrients to (1).
  (3) Add ⅓ volume of ascitic fluid (specific gravity to be 1013 or over).
  (4) Reaction of medium to be between 1.0 and 1.5%.
  (5) Tinge with Kahlbaum's litmus.
  (6) Method of sterilization not given.

The added nutrients employed were:

glucose
lactose
sucrose
maltose
mannitol
arabinose
inulin
raffinose
galactose
dextrin

(b) Harvey prepared a medium as follows:
  (1) Mix one part nutrose with 15 parts ascitic fluid and 35 parts distilled water.
  (2) Raise slowly to boiling temperature with frequent shaking to prevent burning.
  (3) Boil or steam 30 minutes.
  (4) Clarify by the addition of egg and filter thru thick filter paper.
  (5) Add one part (4) at 45°C. to two parts melted infusion agar cooled to 45°C. (see variant (v) medium 1661 for preparation).
  (6) Steam 30 minutes.
  (7) Distribute into test tubes.
  (8) Sterilize.

(c) Harvey cultivated meningococci on a mixture of one part ascitic fluid at 45°C. with three parts melted infusion agar, (see medium 1661, variant (v) for preparation) containing 1.0% glucose at 45°C. Reaction of the agar to be faintly alkaline to litmus.

(d) Harvey cultivated meningococci on a mixture of seven parts sterile ascitic fluid at 45°C., three parts of a sterile standard litmus solution at 45°C., two parts of a sterile 10.0% solution of any desired carbohydrate at 45°C., 0.15 part of a normal NaOH solution at 45°C., and 21 parts of melted infusion agar at 45°C. (See variant (v) medium 1661 for preparation.) The litmus solution was prepared as follows:
  (1) Place in a well stoppered glass bottle, solid commercial litmus 1; 95.0% alcohol 6.
  (2) Shake one daily for seven days.
  (3) Reject the alcohol.
  (4) Add fresh 95.0% alcohol.
  (5) Shake well once daily.
  (6) Repeat the addition of fresh alcohol until the alcohol becomes only lightly tinged with violet on shaking up with the litmus.
  (7) Reject the alcohol.
  (8) Dry the alcohol insoluble residue.
  (9) Make a saturated solution of the dried residue in distilled water.
  (10) Filter.
  (11) Dilute a portion of this saturated solution with distilled water until its tint is a pure blue.
  (12) Add to this pure blue solution very dilute sulphuric acid till the blue color is turned to wine red.
  (13) Add to this wine red solution the saturated solution obtained from the alcohol insoluble residue until the blue color returns.
  (14) Use as a sensitive litmus solution. Sterile 10.0% sugar at 45°C., 2; sterile N/1 sodium hydroxide at 45°C., 0.15; melted nutrient agar at 45°C., 21.

(e) Harvey cultivated *B. acnes* on a medium composed of 2 parts sterile oleic acid, 20 parts sterile ascitic fluid, 0.8 part of a sterile 1.0% neutral red and 100 parts of sterile infusion agar (see medium 1661, variant (v) for preparation).

(f) Torrey and Buckell used the follow-

ing medium to determine fermentation of gonococci:
(1) Prepare sugar free infusion broth.
(2) Add 15.0 g. peptone and 6.6 g. agar to (1) and dissolve.
(3) Adjust to reaction pH = 7.0.
(4) Add brom-thymol-blue in sufficient amount to give fairly deep color.
(5) Tube in 5.0 cc. lots.
(6) Sterilize in autoclave.
(7) To each tube of (6) add 1.0 cc. ascitic fluid free from fermentable substances by prolonged storage in ice box.
(8) Expose a 12.0% solution of any desired carbohydrate to flowing steam at 100°C. for 12 minutes.
(9) Add 1.0 cc. of (8) to each tube of (7).
(10) Incubate 3 days at 37°C. to determine sterility.

Author reported that with glucose, medium below surface growth was changed from bluish green to yellow in 18 to 48 hours. In case of unfermented sugars color remained unchanged or a bluish tinge developed.

References: Plotz, Olitsky and Baehr (1915 p. 10), Elser and Huntoon (1909 p. 407), Harvey (1921–22 pp. 83, 84, 87), Torrey and Buckell (1922 p. 145).

1926. Gilbert and Humphrey's Tellurite Serum Agar

Constituents:

1. Beef infusion agar (1.5%)	1000.0 cc.
2. Serum, horse (5.0%)	50.0 cc.
3. Glucose (1.0%)	10.0 g.
4. Potassium tellurite 1% solution (0.58% on test) (1%)	10.0 cc.

Preparation:
(1) Melt sterile beef infusion agar and cool to 50°C.
(2) Add 50.0 cc. of sterile horse serum, 10.0 cc. of a sterile 20% glucose solution, heated to 50°C.
(3) Weigh out 250 mgm. K_2TeO_3 on a chemical balance. Grind to a very fine powder.
(4) Add about 10.0 cc. of water. Mix well.
(5) Allow the undissolved portion to settle out and pour off the supernatant fluid.
(6) Add more water to the residue and grind again. Combine the two portions.
(7) Rinse the mortar with the remainder of the 25.0 cc. of water.
(8) Filter thru paper.
(9) Heat sterile (8) to 50°C. and add to (2).
(10) Mix (9) thoroly and insert in a sterile siphon.
(11) Dispense in Petri dishes.
(12) Incubate the plates to test sterility.

Sterilization: Method of sterilization of agar, serum or glucose not given. Sterilize the potassium tellurite solution by filtration thru a Mandler.

Use: Isolation of diphtheria bacilli. Diphtheria colonies white, or white about the periphery with grey centers. Staphylococci are black.

Reference: Gilbert and Humphreys (1926 p. 149).

1927. Scholtz's Ascitic Fluid Agar

Constituents:
1. Infusion agar.
2. Ascitic fluid.

Preparation:
(1) Prepare infusion agar.
(2) Mix (1) with ascitic fluid (pericardic or hydrocelenic fluid may be used) in the ratio of 3 to 1.

Sterilization: Not specified.

Use: Cultivation of gonococci.

Variants:
(a) Swartz and Davis used the following medium to cultivate gonococci. Have the medium at body temperature when inoculating. Following inoculation hold the tubes horizontally so that the agar slant is uppermost. Holding the tubes by the butt, pass the tubes thru the flame three times longitudinally and cork quickly.
  (1) Prepare a 2.0% beef (or veal) infusion agar in the usual manner.
  (2) Adjust (1) to pH 7.6 (pH 7.4 after autoclaving) and sterilize in the autoclave.
  (3) Mix one part sterile ascitic fluid

with two parts sterile melted (2). (Pleuritic or hydrocele fluid may be used instead of ascitic fluid.)
- (4) Replace the cotton stoppers with sterile corks.

(b) Harvey prepared a similar medium as follows:
- (1) Mix one part ascitic fluid with two parts infusion agar (see variant (v) medium (1661) for preparation).
- (2) Sterilize in the water bath 30 minutes at 56°C. on five successive days.
- (3) Test sterility before use by incubation 48 hours.

(c) Schwartz (Stitt) gave the following method of preparation:
- (1) Prepare beef or veal infusion agar in the ordinary manner.
- (2) Adjust (1) to pH = 7.6 phenolsulphonephthalein being used as an indicator in the hot agar.
- (3) Cool to 50°C.
- (4) Add the whites of three fresh eggs to each liter of agar.
- (5) Start with a low flame, boil for 10 minutes.
- (6) Strain thru cloth and filter thru paper.
- (7) Tube in 5.0 to 6.0 cc. quantities and autoclave at 10 pounds pressure on 3 successive days.
- (8) Sterilization reduces the pH to 7.4.
- (9) Add sterile ascitic, pleuritic or hydrocele fluid to each tube. One part fluid to two parts agar.
- (10) Seal the tubes with sterile rubber stoppers and slant.
- (11) Cork the tubes and store in the incubator.
- (12) Discard all contaminated tubes.
- (13) Inoculate when the medium is at body temperature.
- (14) After inoculation hold the tube horizontal by the butt with the agar slant up. Pass longitudinally thru the Bunsen flame about three or four times and cork quickly. This reduces the pressure within the tube.
- (15) The agar tubes should have about 0.5 cc. of water of condensation in the lower angle of the slant.

(d) Mulsow used the following medium for the isolation of gonococci:
- (1) Infuse one pound of ground lean beef in 500.0 cc. of distilled water over night in the ice box.
- (2) Dissolve 10.0 g. peptone in the juice while cool.
- (3) Add 500.0 cc. of a 3.0% agar solution, melted and cooled to 60°C., to (2).
- (4) Adjust to +0.9 to phenolphthalein.
- (5) Before the agar has cooled sufficiently to harden, it is heated in the autoclave at 15 pounds pressure for 25 minutes.
- (6) Filter thru moistened cotton and canton flannel.
- (7) Distribute in 100.0 cc. lots in flasks.
- (8) When ready for use, sterile (7) is melted and cooled to 60°C. and added to 50.0 cc. of ascitic fluid. (0.5 g. of any carbohydrate (levulose or maltose) may be added along with 1 cc. of a 0.04% solution of brom cresol purple.)
- (9) Pour in sterile plates.
- (10) Final reaction −1.2 to phenolphthalein or pH = 6.8.

References: Scholtz (1899 p. 5), Schwartz and Davis (1920 p. 1125), Harvey (1921–22 p. 83), Stitt (1923 p. 45), Mulsow (1925 p. 421).

1928. Kiefer's Ascitic Fluid Agar (Abel)

Constituents:

1. Water	1000.0 cc.
2. Meat	500.0 g.
3. Agar (3.5%)	35.0 g.
4. Peptone (5.0%)	50.0 g.
5. Glycerol (2.0%)	20.0 g.
6. NaCl (0.5%)	5.0 g.
7. Ascitic fluid	1000.0 cc.

Preparation:
- (1) Chop 500.0 g. of fat free meat and add to a liter of water at 50°C.
- (2) Keep at 50°C. for 30 minutes and then boil for 30 to 45 minutes.
- (3) Filter or strain the fluid from the meat.
- (4) Make up the fluid to 1 liter.
- (5) Dissolve 3, 4, 5 and 6 in (4).
- (6) Neutralize.
- (7) Cool to 50°C.

(8) Mix (7) with an equal volume of ascitic fluid.
(9) Distribute in tubes or plates.

Sterilization: Not specified.

Use: Cultivation of gonococci. Author reported that if the ascitic fluid be strongly alkaline mix with unneutralized or strongly acid agar. The final medium should be slightly alkaline.

Variants: Harvey prepared a similar medium as follows:
(1) Mix equal parts pure neutral glycerol and ascitic fluid, (pleuritic fluid, hydrocele fluid, ovarian fluid, milk, urine, etc., may be used instead).
(2) Leave until sterile on culture test.
(3) Add (2) to sterile infusion agar (see variant (v) medium 1661 for preparation).

References: Abel (1912 p. 162), Harvey (1921–22 p. 83).

1929. Torrey and Buckell's Urine Ascitic Fluid Agar

Constituents:

1. Distilled water	2000.0 cc.
2. Veal, fat-free	1250.0 g.
3. Peptone (Difco)	20.0 g.
4. Urine, fresh	40.0 cc.
5. NaCl	10.0 g.
6. Glycerol	40.0 cc.
7. Flaked agar	36.0 g.
8. Ascitic fluid	1000.0 cc.
9. Iodin-green	

Preparation:
(1) Place finely chopped lean veal in water and bring slowly to a boil.
(2) Allow to simmer 20 minutes with occasional stirring.
(3) Strain thru cotton flannel.
(4) Cool and remove the fat.
(5) Place in double boiler over a saturated brine bath and raise the temperature to about 60°C.
(6) Add 3, 4, 5, 6 and 7 and boil 45 minutes to dissolve materials.
(7) Adjust reaction to pH = 6.9, using 10.0% Na_2CO_3.
(8) Boil 30 minutes longer.
(9) Remove from brine and add distilled water to 2 liters.
(10) Filter thru canton flannel and tube in 10.0 cc. amounts.
(11) In preparing plates 5.0 cc. of ascitic fluid free from bile and 0.5 cc. of a 1:3000 dilution of iodin-green (Grübler) are added to each tube of sterile melted (11) just before pouring.
(12) Final reaction pH = 7.2.

Sterilization: Autoclave (10) at about 12 pounds pressure for 10 minutes.

Use: Isolation of gonococci. Authors reported that after 48 hours gonococci colonies were semi-translucent and had raised or even indented edges. They stood out from the medium. Centers somewhat thickened. If used in slants use 40.0 g. agar.

Variants: The authors omitted the iodin-green.

Reference: Torry and Buckell (1922 p. 125).

1930. Watabiki's Whey Ascitic Fluid Agar

Constituents:

1. Whey	200.0 cc.
2. Ascitic fluid (5.0%) agar	
3. Nutrose (0.3%) agar	400.0 cc.
4. Urea	2.0 g.

Preparation:
(1) Warm 200.0 cc. of cow's milk to 60°C.
(2) Add 5.0% ascitic agar drop by drop. (Composition or method of preparation not given) the milk being shaken to cause precipitation of the casein.
(3) Filter thru filter paper.
(4) To filtrate add 10.0% caustic soda to slightly alkaline.
(5) Add urea (2.0 g.).
(6) Mix sterile (5) with 0.3% nutrose agar. (Composition or method of preparation not given.) One part fluid (5) to 2 parts agar at 45°C.
(7) Slant or plate as desired.
(8) Incubate to insure sterility.

Sterilization: Sterilize (5) by heating at 60°C. for 30 minutes on each of three successive days.

Use: Cultivation of gonococci. Author reported that the medium was not as satisfactory as blood agar or blood broth. Ordinary broth or peptone water may be mixed with the fluid or liquid medium.

Reference: Watabiki (1916 p. 734).

1931. Esch's Maltose Ascitic Fluid Agar

Constituents:

1. Infusion agar	750.0 cc.
2. Peptone, Witte (1.0%)	7.5 g.

3. Maltose	10.0 g.
4. Ascitic fluid	250.0 cc.

Preparation:

(1) Prepare infusion agar, containing 1.0% Witte's peptone.

(2) Mix 75 parts (2) with 25 parts ascitic fluid.

(3) Add 1% maltose to (2).

Sterilization: Not specified.

Use: Cultivation of meningococci.

Reference: Esch (1909 p. 152).

1932. Steinschneider's Hydrocele Fluid Agar

Constituents:

1. Infusion agar	300.0 cc.
2. Hydrocele fluid	100.0 cc.

Preparation:

(1) Mix three parts sterile infusion agar with one part sterile liquid hydrocele fluid.

(2) Pour into sterile Petri dishes.

Sterilization: Method not given.

Use: Cultivation of gonococci.

Reference: Steinschneider (1890 p. 533).

1933. Heiman's Pleuritic Serum Agar

Constituents:

1. Infusion agar (2.0%)	200.0 cc.
2. Pleuritic serum	100.0 cc.
3. Peptone (1.0%)	2.0 g.
4. NaCl (0.5%)	1.0 g.

Preparation: (1) Add one part sterilized liquid pleuritic (chest) serum to 2.0% agar containing 1.0% peptone and 0.5% NaCl.

Sterilization: Sterilize on 9 successive days at 65°C.

Use: Cultivation of gonococci.

Reference: Heiman (1896 p. 888).

1934. Lentz and Tietz's Malachite Green Bile Agar (Klimmer)

Constituents:

1. Meat infusion	2000.0 cc.
2. Agar	60.0 g.
3. Peptone	20.0 g.
4. NaCl	10.0 g.
5. Bile	30.0 cc.
6. Malachite Green I (Höchst)	

Preparation:

(1) Soak 60.0 g. of agar in 2000.0 cc. of meat water.

(2) Boil for 3 hours.

(3) Dissolve 3, 4 and 5 in 250.0 cc. of water by heating slightly.

(4) Mix (3) and (2).

(5) Neutralize to litmus or adjust to 1.0 to 1.5 acid to phenolphthalein.

(6) Boil for an hour.

(7) Readjust the reaction if necessary.

(8) Distribute in flasks.

(9) The reaction of the sterile agar when ready for use should be 1.8%.

(10) Melt sterile (9) and add 30.0 cc. of sterile beef bile and 1.0 cc. of a solution of 1.0 g. malachite green I (Höchst) in 60.0 cc. distilled water. It is preferable to mix 20.0 cc. of agar with 0.05, 0.1, 0.15, 0.2, etc., cc. of a 2.0% malachite green solution and pour into plates. Inoculate with colon and typhoid bacilli and use that dilution of malachite green that inhibits the colon organisms but not the typhoid organisms. Add a corresponding amount of malachite green and bile to the 100.0 cc. of agar.

Sterilization: Method of sterilization of (8) not given.

Use: Cultivation of colon-typhoid group.

Reference: Klimmer (1923 p. 212).

1935. Lentz and Tietz's Malachite Green Nutrose Bile Agar (Klimmer)

Constituents:

1. Meat infusion	2000.0 cc.
2. Agar	60.0 g.
3. Peptone	20.0 g.
4. NaCl	10.0 g.
5. Nutrose	20.0 g.
6. Bile, beef	30.0 cc.
7. Malachite green	

Preparation:

(1) Soak 60.0 g. of agar in 2000.0 cc. of meat water.

(2) Boil for three hours.

(3) Dissolve 3, 4 and 5 in 250.0 cc. of water by heating slightly.

(4) Mix (3) and (2).

(5) Neutralize to litmus or adjust to 1.0 to 1.5 acid to phenolphthalein.

(6) Boil for an hour.

(7) Readjust the reaction if necessary.

(8) Distribute in flasks.

(9) The reaction of the sterile agar when ready for use should be a −3.5%.

(10) Melt sterile (9) and add 3.0 cc. of

sterile beef bile and 1.0 cc. of a solution of 1.0 g. of malachite green I (Höchst) in 60.0 cc. distilled water. It is preferable to mix 20.0 g. of agar with 0.05, 0.1, 0.15, 0.2, etc., cc. of a 2.0% malachite green solution and pour into plates. Inoculate with colon and typhoid bacilli and use that dilution of malachite green that inhibits the colon organism but not the typhoid organism. Add a corresponding amount of malachite green and bile to the 100.0 cc. of agar.

Sterilization: Method of sterilization of (8) not given.

Use: Cultivation of colon-typhoid group.

Reference: Klimmer (1923 p. 212).

1936. v. Drigalski and Conradi's Crystal Violet Nutrose Agar

Constituents:

1. Water	2000.0 cc.
2. Beef	3.0 lbs.
3. Peptone (Witte)	20.0 g.
4. Nutrose	20.0 g.
5. NaCl	10.0 g.
6. Agar	60.0 g.
7. Litmus solution	
8. Lactose	30.0 g.
9. Crystal violet (B. Höchst)	

Preparation:

(1) Allow 2 liters of water to stand with 3 pounds of finely chopped beef over night.
(2) Filter off the meat water and boil one hour.
(3) Filter.
(4) Add 3, 4, 5 and boil one hour.
(5) Filter.
(6) Add 60.0 g. of fine agar, boil 3 hours (or autoclave one hour) and make slightly alkaline to litmus.
(7) Filter and boil 3 minutes.
(8) Boil 260.0 g. of litmus solution (prepared according to Kubel and Tiemann, method or reference not given) for 10 minutes and add 30.0 g. c.p. lactose.
(9) Boil (8) for 15 minutes.
(10) Mix hot (9) and (7).
(11) Again adjust to slight alkalinity.
(12) Add 4.0 cc. of a hot, sterile 10.0% water free soda solution.
(13) Add 20.0 cc. of a freshly prepared solution of 0.1 g. Crystal violet B Höchst in 100.0 cc. sterile warm distilled water.
(14) Pour into plates.

Sterilization: Not specified.

Use: Differentiation of colon-typhoid group. Authors reported that coli colonies were red, deep red and wine red. *Bact. typhi* colonies were small, of bluish color and glossy.

Variants: The following authors prepared the medium as indicated:

(a) Frost used a sugar free infusion broth and specified that the medium be sterilized for 20 minutes on 3 successive days.

(b) Harris (Heinemann).
  (1) Dissolve 20.0 g. nutrose and 40.0 g. agar in 2000.0 cc. glucose free infusion broth.
  (2) Neutralize (1) to phenolphthalein.
  (3) Autoclave at 120° for 5 minutes.
  (4) Clarify with the white of eggs and filter.
  (5) Add 30.0 g. lactose, 260.0 cc. of litmus solution and 20.0 cc. of a 0.1% aqueous solution of Crystal violet.
  (6) Tube.
  (7) Sterilize in the Arnold.

(c) Wesbrook.
  (1) Add 680.0 g. finely chopped lean beef to 1000.0 cc. water and place in cold for 24 hours.
  (2) Express the juice and make up to one liter.
  (3) Coagulate albumin either by vigorous boiling 10 minutes or by heating at 120°C. in the autoclave.
  (4) Filter.
  (5) Dissolve 10.0 g. Witte's peptone, 10.0 g. nutrose and 5.0 g. NaCl in (4).
  (6) Heat in autoclave at 120°C. for 30 minutes or boil vigorously for 15 minutes.
  (7) Render slightly alkaline to litmus paper.
  (8) Filter.
  (9) Add 30.0 g. agar to (8).
  (10) Heat in autoclave at 120°C. for 30 minutes, or over flame until agar is dissolved.

(11) Render slightly alkaline to litmus while hot if necessary.
(12) Filter thru glass wool into a sterile vessel.
(13) To 130.0 cc. of litmus solution (Kubel and Tiemann) add 15.0 g. c.p. lactose.
(14) Boil (13) for 10 minutes.
(15) Mix (14) and (12) while hot.
(16) Render slightly alkaline to litmus paper if necessary.
(17) To (16) add 2.0 cc. of hot sterile solution of 10.0% sodium hydrate in distilled water, and 10.0 cc. of fresh solution of Höchst's crystal violet (0.1 g. to 100.0 cc. of sterile water).
(18) Pour in sterile Petri dishes. Glass covers of Petri dishes may be discarded and use porous clay covers.

(d) Reitz used the following medium to study the bacteriology of butter and butter examination:
(1) Chop 3 pounds of horse meat to small pieces.
(2) Pour 2 liters of water over (1) and allow to stand until the next day (in ice box).
(3) Press the water from the meat.
(4) Add 2.0% Witte peptone, 2.0% nutrose and 0.5% NaCl to the meat water and boil an hour.
(5) Filter thru a linen towel and add 3.0% agar.
(6) Boil in the steamer for 3 hours and filter thru linen in the steamer.
(7) Prepare 300.0 cc. of a 15.0% Kahlbaum's litmus solution.
(8) Add 30.0 g. lactose to (7) and boil for 15 minutes.
(9) Mix hot (8) and hot (6).
(10) Add 10.0% soda solution until the reaction is slightly alkaline to phenolphthalein.
(11) To (10) add 6.0 cc. of a sterile warm 10.0% soda solution and 20.0 cc. of a fresh solution of 0.1 g. crystal violet (Höchst c.p.) in 100.0 g. sterile distilled water.
(12) Distribute in 200.0 cc. lots in sterile Erlenmeyer flasks.
(13) Final sterilization not specified.

(e) Schmitz used the following medium as an elective medium for typhoid bacilli:
(1) Obtain 7 or 8 liters of blood from the slaughter house.
(2) Pour off the serum from the clot.
(3) Add a double amount of water to the clot.
(4) Boil the mixture. Stir constantly but take care not to break up the clot too much or filtration will be too difficult. Boil for 5 to 10 minutes.
(5) Filter thru a towel, then thru cotton or glass wool.
(6) Add peptone nutrose, in the usual amount (exact amount not specified) and 3.0% agar.
(7) When the agar has been completely dissolved, add the serum that had been previously poured from the blood and that had been allowed to stand undisturbed.
(8) Boil for a few minutes.
(9) Place in a flask with a patented stopper and heat for one hour in streaming steam.
(10) Allow to cool slowly so that the sediment and turbidity may settle to the bottom. Use only the top clear medium.
(11) If caffeine is desired in the medium prepare a solution of caffeine in water so that 1.0 cc. of solution contains 0.1 g. caffeine. Sterilize and add 6.0 cc. of this solution to 100.0 cc. of the agar.

(f) Roddy.
(1) Mix 750.0 g. of finely minced beef with 1000.0 cc. of water.
(2) Place in a shallow dish in the ice ice box over night.
(3) Skim off the fat.
(4) Boil for one hour.
(5) Filter.
(6) Bring this volume to 1000.0 cc.
(7) Add 10.0 g. Witte's peptone, 10.0 g. nutrose and 5.0 g. $CaCl_2$ to (6).
(8) Boil.
(9) Add 30.0 g. agar and boil until solution is complete.
(10) Make faintly alkaline to litmus.
(11) Autoclave for one hour.
(12) Filter thru paper.

(13) Autoclave for 15 minutes.
(14) Add 150.0 cc. of Kahlbaum's litmus solution and 15.0 g. lactose to (13).
(15) Sterilize in the Arnold steamer for 15 minutes.
(16) Add 10.0 cc. of a hot 0.1% solution of crystal violet (B. Höchst).

(g) Harvey.
(1) Mince finely fat free beef.
(2) Add 15.0 cc. to 2000.0 cc. water.
(3) Heat the mixture 20 minutes at a temperature not exceeding 50°C. over a free flame.
(4) Skim off fat floating on the surface.
(5) Raise the temperature to boiling point.
(6) Boil 10 minutes.
(7) Pour the meat and fluid on to a wet, thick, clean cloth.
(8) Filter the fluid collected thru thick, well-wetted, filter paper.
(9) Add to the filtrate, peptone 20.0 g., nutrose 20.0 g., sodium chloride 10.0 g. and see that the mixture is alkaline to litmus.
(10) Steam 45 minutes.
(11) Add 60.0 g. prepared fiber agar (see medium 1401).
(12) Steam gently 2½ hours or heat 45 minutes at 118°C. to bring the agar thoroly into solution.
(13) Make faintly alkaline to litmus.
(14) Filter, while hot, thru well-wetted, thick filter paper by placing filter funnel, stand and receptacle for filtrate in the steam sterilizer and steaming until filtration is complete.
(15) Boil 300.0 cc. litmus solution 10 minutes and dissolve in it 30.0 g. chemically pure lactose.
(16) Boil the lactose litmus solution 15 minutes.
(17) Mix hot lactose litmus solution and the melted nutrose agar.
(18) Make faintly alkaline if necessary by the addition of 10.0% sodium hydroxide.
(19) Add 4.0 cc. hot 10.0% sodium hydroxide and 20.0 cc. hot sterile 0.1% c.p. crystal violet.
(20) Distribute in flasks.
(21) Pour large plates for use, with a layer of medium about 4 mm. thick.
(22) Place the plates so made, in the inverted position in the incubator without their covers and resting on a support.

(h) Klimmer reported that tropon could be used instead of nutrose, that mannitol or maltose might be used instead of lactose, and a 1.0% Eichloff's solution may be substituted for meat infusion. (Eichloff is a skim milk preparation obtained from a food factory.)

References: v. Drigalski and Conradi (1902 p. 291), Frost (1903 p. 342), Heinemann (1905 p. 130), Westbrook (1905 p. 319), Reitz (1906 p. 721), Abel (1912 pp. 128, 129), Kolle and Wasserman (1912 p. 415), Schmitz (1915 p. 307), Roddy (1917 p. 44), Tanner (1919 p. 62), Ball (1919 p. 81), Giltner (1919 p. 387), Bezançon (1920 p. 342), Levine (1921 p. 116), Abbott (1921 p. 523), Harvey (1921–22 p. 90), Klimmer (1923 p. 208).

1937. Galli-Valerio's Congo Red Nutrose Agar

Constituents:

1. Water	2000.0 cc.
2. Meat, horse	1000.0 g.
3. Peptone	20.0 g.
4. Nutrose	20.0 g.
5. NaCl	10.0 g.
6. Agar	60.0 g.
7. Lactose	30.0 g.
8. Congo red (1.0% soln.)	30.0 cc.

Preparation:
(1) Boil 1000.0 g. of horse meat with 2 liters of water for an hour.
(2) Filter.
(3) Add 3, 4 and 5 to the filtrate and boil for an hour.
(4) Filter.
(5) Add 60.0 g. of agar and heat for an hour in the autoclave.
(6) Add soda solution until the reaction is slightly alkaline to litmus.
(7) Boil again for an hour and filter hot in the sterilizer.
(8) Distribute in 100.0 cc. lots and add 1.5 g. lactose and 30.0 cc. of 1.0%

water solution of congo red to each flask.

Sterilization: Method not given.

Use: Water analysis. Detection of *B. coli*. Author reported that *B. coli* colonies were intensive black (bluish black). *B. typhi*, *B. paratyphi A* and *B*, *B. dysentery*, *Shiga Flexner Y*, *B. enteritidis*, *B. cholerae* did not show this black or dark color. *B. acidi lactici* and *B. lactis-aerogenes* gave the same reaction as *B. coli*.

Reference: Galli-Valerio (1916 p. 136).

1938. Teague and Travis' Eosin Bismark Brown Nutrose Agar

Constituents:

1. Distilled water	2000.0 cc.
2. Chopped beef	2.0 lbs.
3. Peptone (Witte)	20.0 g.
4. NaCl	10.0 g.
5. Nutrose	5.0 g.
6. Sucrose	20.0 g.
7. Eosin 3.0% bluish solution	40.0 cc.
8. Bismark brown 1.0% solution	80.0 cc.
9. Agar	15.0 g.

Preparation:

(1) Soak beef in water in ice chest over night.
(2) Squeeze out fluid.
(3) Heat liquid in Arnold sterilizer and filter thru filter paper.
(4) Neutralize with NaOH and heat again.
(5) Cool and inoculate with *B. coli* and incubate for 2 or 3 days.
(6) Add 3, 4 and 9 to (5).
(7) Clear with egg. Filter.
(8) Add nutrose.
(9) Prepare a 3.0% stock solution of bluish eosin in distilled water. Store in the dark.
(10) Prepare a 1.0% solution of bismark brown in water containing 10.0% alcohol (not soluble to 1.0% in pure water).
(11) To 50.0 cc. of (8) add 1.0% sucrose (0.5 g.), 1.0 cc. of (9) and 2.0 cc. of (10).
(12) Shake well.
(13) Pour into sterile Petri dishes.
(14) Dry by inverting plates in the incubator for 20 to 30 minutes.

Sterilization: Not specified.

Use: To isolate cholera vibrio. Authors reported that cholera vibrio colonies had a dark colored center. Liebig's meat extract may be substituted for infusion.

Reference: Teague and Travis (1916 p. 602).

1939. Gassner's Nutrose Agar

Constituents:

1. Meat infusion	1000.0 cc.
2. Peptone	10.0 g.
3. Nutrose	10.0 g.
4. Agar	30.0 g.
5. NaCl	5.0 g.

Preparation:

(1) Prepare meat infusion.
(2) Dissolve 2, 3, 4 and 5 in 1.
(3) Adjust to slightly alkaline to litmus.

Sterilization: Not specified.

Use: Cultivation of colon-typhoid group.

Reference: Gassner (1916–17 p. 315).

1940. Loeffler's Malachite Green Nutrose Agar (Roddy)

Constituents:

1. Distilled water	500.0 cc.
2. Infusion broth	500.0 cc.
3. Agar	30.0 g.
4. Nutrose	10.0 g.
5. Malachite green (2.0% soln.)	20.0 to 30.0 cc.

Preparation:

(1) Prepare infusion broth.
(2) Mix equal volumes of (1) and distilled water.
(3) Neutralize (indicator not specified).
(4) Add 7.5 cc. of a normal HCl solution.
(5) Dissolve 30.0 g. of shredded agar in (4) by boiling.
(6) Neutralize by the addition of sodium hydrate.
(7) Add 5.0 cc. of normal Na_2CO_3 solution.
(8) Steam for 2 hours.
(9) Add 100.0 cc. of a 10.0% nutrose solution.
(10) When ready for use, liquefy sterile (9) and add between 2.0 and 3.0 cc. of a 2.0% aqueous solution of malachite green (Höchst 120) to each 10.0 cc. of medium.

Sterilization: Sterilize (9) in streaming steam for 15 minutes on each of 2 successive days.

Use: General culture medium.

Reference: Roddy (1917 p. 44).

1941. Schmitz's Nutrose Blood Clot Infusion Agar

Constituents:

1. Water	1000.0 cc.
2. Blood clot	500.0 g.
3. Peptone	
4. Nutrose	
5. Agar (3.0%)	30.0 g.

Preparation:

(1) Obtain 7 or 8 liters of blood from the slaughter house.

(2) Pour off the serum from the clot.

(3) Add a double amount of water to the clot.

(4) Boil the mixture. Stir constantly but take care not to break up the clot too much or filtration will be too difficult. Boil for 5 to 10 minutes.

(5) Filter thru a towel, then thru cotton or glass wool.

(6) Add peptone nutrose, in the usual amount (exact amount not specified) and 3.0% agar.

(7) When the agar has been completely dissolved, add the serum that had been previously poured from the blood and that has been allowed to stand undisturbed.

(8) Boil for a few minutes.

(9) Place in a flask with a patented stopper and heat for one hour in streaming steam.

(10) Allow to cool slowly so that the sediment and turbidity may settle to the bottom. Use only the top clear medium.

Sterilization: Not specified.

Use: Selective medium for typhoid bacilli.

Variants: The author gave the following variants:

(a) If caffeine is desired in the medium prepare a solution of caffeine in water so that 1.0 cc. of solution contains 0.1 g. caffeine. Sterilize. Add 6.0 cc. of this solution to 100.0 cc. of the agar.

(b) (1) Mix 15 parts lactose and 1 part congo red.

(2) To 100.0 cc. of the sterile melted agar, add 1.5 g. (1).

(3) Pour the red medium into sterile Petri dishes.

(4) If caffeine is desired in the medium prepare a solution of caffeine in water so that 1.0 cc. of solution contains 0.1 g. caffeine. Sterilize and add 6.0 cc. of this solution to 100.0 cc. of the agar.

Reference: Schmitz (1915 pp. 307, 315).

1942. Harvey's Milk Agar

Constituents:

1. Infusion agar.
2. Milk.

Preparation: (1) Add 3.0 cc. of sterilized milk to a tube of melted infusion agar (see variant (v) medium 1661 for preparation).

Sterilization: Not specified.

Use: General culture medium.

Variants: Harvey gave the following variants:

(a) Use litmus milk instead of plain milk.

(b) Mix equal parts of sterilized milk at 45°C. and melted infusion agar at 45°C. (See variant (v) medium 1661 for preparation).

Reference: Harvey (1921–22 p. 95).

1943. Lubenau's Caffeine Whey Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Beef	500.0 g.
3. NaCl	10.0 g.
4. Peptone	60.0 g.
5. Agar	40.0 to 60.0 g.
6. Litmus whey	900.0 cc.
7. Caffeine	6% solution

Preparation:

(1) Boil 500.0 g. of finely chopped lean beef with one liter distilled water for 30 minutes.

(2) Filter and make up to one liter.

(3) Add 3, 4, and 5 to (2) and dissolve by boiling in a salt water bath.

(4) Neutralize to litmus.

(5) Boil and filter.

(6) Add 900.0 cc. of a sterile litmus whey,

(preparation not given) to hot sterile (5).

(7) Mix thoroly and allow to cool.

(8) Add 110.0 cc. of a sterile 6.0% caffeine solution to (7). Mix well.

(9) Pour in plates.

Sterilization: Method of sterilization of (5) not given.

Use: Detection of typhoid bacteria. Authors reported that typhoid colonies were colorless.

Reference: Lubenau (1907 p. 248).

1944. Harvey's Whey Infusion Agar

Constituents:

1. Infusion agar.
2. Whey.

Preparation:

(1) Mix 1000.0 cc. of fresh milk with 5.0 cc. of 1-4 HCl.

(2) Keep at 37°C.

(3) Filter.

(4) Make the filtrate neutral to litmus with sodium hydroxide.

(5) Steam 2 hours.

(6) Estimate and adjust the reaction.

(7) Filter.

(8) Mix one part sterile (7) with two parts sterile infusion agar (see variant (v) medium 1661 for preparation).

Sterilization: Sterilize (7) at 100°C.

Use: Cultivation of gonococci.

Reference: Harvey (1921-22 p. 95).

1945. Schloffer's Urine Infusion Agar

Constituents:

1. Infusion agar (2.0%)	200.0 cc.
2. Urine	100.0 cc.

Preparation:

(1) Prepare a 2.0% infusion agar.

(2) Obtain urine under aseptic conditions.

(3) Mix two parts sterile agar with one part sterile urine.

(4) Distribute into sterile plates, tubes or flasks.

Sterilization: Method of sterilization of infusion agar not given.

Use: Cultivation of diphtheria bacilli. Author reported that in pure culture colonies were grayish-white to white, quite luxuriant and rather dry. Ghon-Schlagenhaufer used a similar medium for the isolation of gonococci.

Variants: Schloffer reported that urine may be collected in sterile flasks and then heated to 70 or 80°C. to sterilize.

References: Schloffer (1893 p. 657), Ghon-Schlagenhaufer (1893 p. 619).

1946. Ficker's Glycerol Sputum Agar

Constituents:

1. Infusion agar (2.0%)	200.0 cc.
2. Sputum	100.0 cc.
3. Glycerol	6.0 cc.

Preparation:

(1) Prepare neutral 2.0% infusion agar.

(2) Mix 2 parts sterile (1) with 1 part sterile tuberculous or bronchitus sputa.

(3) Add 2.0% glycerol.

(4) Cool to 45°, mix thoroly and allow to solidify.

Sterilization: Sterilize tuberculous or bronchitus sputa for an hour on each of 3 successive days in the steamer. Method of sterilization of (1) not given.

Use: Cultivation of tubercle bacilli.

Reference: Ficker (1900 p. 508).

1947. Szabōky's Sputum Lung Infusion Agar

Constituents:

1. Water	200.0 cc.
2. Lung infusion agar	50.0 cc.
3. Sputum	50.0 g.
4. NaCl	0.5 g.
5. Glucose	0.5 g.
6. Glycerol	12.0 g.
7. Peptone	1.0 g.

Preparation:

(1) See medium 1746 for preparation of lung infusion agar.

(2) Boil slowly for one hour.

(3) Filter while hot.

(4) Adjust to neutral or $\frac{1}{8}$% acid.

Sterilization: Sterilize on 3 successive days in streaming steam.

Use: Cultivation of tubercle bacilli. Author reported that growth appeared in two days with neutral or $\frac{1}{8}$% acid medium.

Reference: Szabōky (1906 p. 657).

1948. Costa and Boyer's Gum Infusion Agar

Constituents:

1. Water	
2. Beef	500.0 g.
3. NaCl	5.0 g.
4. Peptone	20.0 g.

5. Agar	30.0 g.
6. Gum tragacanth	10.0 g.

Preparation:

(1) Macerate 500.0 g. of finely chopped beef in 1200.0 cc. water at 37° for 5 or 6 hours.
(2) Press thru a linen cloth.
(3) Add 3 and 4 and boil.
(4) Filter on a wet filter paper.
(5) Neutralize with soda using litmus and then add 7.0 cc. of normal soda solution to a liter of medium.
(6) Pulverize the gum tragacanth in a large dry glass mortar by means of a pestile.
(7) Carefully mix (6) with (5), so as not to obtain lumps or a gum. Mix the bouillon thoroly after each addition of (6).
(8) Add the mixture to a 1.5 or 2 liter flask and add the agar.
(9) Heat at 115° for 30 minutes to dissolve the agar.
(10) Filter while hot.
(11) Distribute.

Sterilization: Sterilize at 115° for 300 minutes.

Use: Isolation of gonococcus. Cultivation of pasteurella and salmonella.

Variants:

(a) To prepare a semi-solid agar use only 3.0 g. agar instead of 30.0 g.
(b) van Saceghem recommended the use of gum arabic instead of gum tragacanth in this medium. When gum arabic is used he obtained vigorous growth of pasteurella and salmonella also.

References: Costa and Boyer (1922 p. 857), van Saceghem (1923 p. 968).

1949. Capaldi's Egg Yolk Agar

Constituents:

1. Nutrient agar.
2. Egg yolk.

Preparation:

(1) Break an egg, separate the white from the yolk and place the yolk in a sterile Petri dish.
(2) Prepare nutrient agar.
(3) Place a glowing glass rod on a spot on the covering of the egg yolk. (This sterilizes the covering of the naturally sterile yolk.)
(4) Melt sterilized agar and cool to 45°C.
(5) Remove 3–4 loops (exact amount not given) of egg yolk thru the portion of covering of the yolk that had been sterilized and add to tube of (4).
(6) Mix thoroly and plate or slant.

Sterilization: Method not given.

Use: Culture and diagnosis of diphtheria. Also used to cultivate tubercle bacilli.

Variants: Bezançon prepared a similar medium as follows:

(1) Place an egg in boiling water for several minutes. This coagulates the white.
(2) Sterilize one end of the shell and make a small opening into the egg.
(3) Remove the yolk from the egg by asparating with a sterile pipette.
(4) Add the yolk (amount not specified) to sterile melted agar.
(5) Mix thoroly.
(6) Slant.

References: Capaldi (1896 p. 801), Heinemann (1905 p. 130), Tanner (1919 p. 58), Bezançon (1920 p. 121).

1949a. Tulloch's Ox Heart Infusion Pea Flour Agar

Constituents:

1. Water	600.0 cc.
2. Peptone (Bacto)	10.0 g.
3. KCl	0.38 g.
4. $CaCl_2$	0.44 g.
5. $NaHCO_3$	0.18 g.
6. Na_2HPO_4 (crystalline)	5.0 g.
7. Agar	25.0 g.
8. Heart, ox	500.0 g.
9. Pea extract	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1 by steaming (500.0 cc. of Douglas' trypsinized broth and 100.0 cc. of Cole's trypamine may be used instead of 600.0 cc. of Bacto peptone solution).
(2) Soften 25.0 g. of fiber agar by soaking in 0.25% acetic acid for 15 minutes. Wash the agar free from acid and squeeze thru lint to get rid of as much water as possible.
(3) Heat (1) and (2) on a water bath for 20 to 30 minutes. Then boil over a free flame for 15 minutes to complete the solution of the agar.
(4) Adjust to pH 7.6 and keep at 56°C.

(5) Add 500.0 cc. of water to 500.0 g. of fresh ox heart and immerse in a 56°C. water bath shaking the container frequently.
(6) When the extract has reached a temperature of 39 to 42°C. place on a 37°C. water bath and allow to extract for 2 hours.
(7) Strain thru butter muslin.
(8) Add (7) to (4) in five 100.0 cc. lots allowing 5 minutes between each addition. Keep the agar at 56°C.
(9) Heat (8) after all the infusion has been added on a water bath for 20 minutes.
(10) Adjust to pH 7.6.
(11) Cool to 56°C. and add the whites of 2 eggs.
(12) Heat on a water bath for 20 minutes.
(13) Pass thru butter muslin and filter thru English Chardin paper at 55°C.
(14) Adjust to pH 7.6.
(15) Transfer, for storage into bottles of 200.0 cc. capacity, each of which contains 10.0 to 15.0 cc. of pea extract.

Sterilization: Sterilize for 15 minutes at 100°C. It is necessary to have the steamer at this temperature before placing the medium in the sterilizer. The medium must not be over-heated, especially after the addition of the heart extract, hence all glassware, butter muslin, paper, etc. are to be steamed before use.

Use: Culture medium for gonococci.

Reference: Tulloch (1922 p. 348).

1950. Nastukoff's Egg Yolk Nutrient Agar (Recktsamer)

Constituents:

1. Distilled water	1000.0 cc.
2. Egg yolk	100.0 g.
3. NaOH (10%)	0.5 cc.
4. Nutrient agar	

Preparation:

(1) Separate the yolk of egg from the white by Bunge's method (allow the whites to roll from a blotting paper containing the egg).
(2) Add 0.5 cc. of a 10% NaOH solution and 100.0 cc. of egg yolk to 1000.0 cc. distilled water.
(3) Prepare nutrient agar.
(4) Mix equal parts of (2) and (3).
(5) Evaporate to ½ the volume by boiling.
(6) Allow to stand two hours in the steamer.
(7) Filter the thick liquid thru a hot water funnel.
(8) Distribute into sterile test tubes.

Sterilization: Sterilize in the steamer.

Use: Cultivation of diphtheria, cholera influenza and other pathogenic forms.

Variants: Besson prepared a similar medium as follows:

(1) Collect egg yolk under aseptic conditions.
(2) Mix one volume of (1) with 3 volumes of sterile water.
(3) Melt tubes of nutrient agar.
(4) Add 2.0 cc. of (2) to each tube of (3) under aseptic conditions.
(5) Slant.

References: Nastiukoff (1893 # 33 and 34), Besson (1920 p. 55).

1951. Bezançon and Griffon's Glycerol Egg Yolk Agar

Constituents:

1. Nutrient agar	100.0 cc.
2. Glycerol	6.0 g.
3. Egg yolk	50.0 cc.

Preparation:

(1) Prepare nutrient agar.
(2) Add 6 parts of glycerol to 100 parts (1).
(3) Tube.
(4) Melt sterile (3) and cool to 50°C.
(5) To each tube add one-half the volume of sterile egg yolk under aseptic conditions.
(6) Mix thoroly and slant to cool.

Sterilization: Method of sterilization of (3) not given.

Use: Cultivation of tubercle bacilli.

References: Bezançon and Griffon (1903 p. 603), Besson (1920 p. 55).

1952. Pergola's Tellurite Egg Yolk Agar

Constituents:

1. Nutrient agar (1.7%)	100.0 cc.
2. Potassium tellurite (1.0% soln.)	2.0 cc.
3. Egg yolk	1

Preparation: Details of method of preparation not given in the abstract.

Sterilization: Method not given.

Use: Diagnosis of diphtheria.

Reference: Pergola (1918 p. 101) taken from (1919 p. 57).

1953. Lipschütz's Egg Albumin Agar

Same as medium 953 but the bouillon is solidified by the addition of 1.0% agar.

1954. Krumwiede, Pratt and Grund's Egg Albumin Agar

Constituents:

1. Water	150.0 cc.
2. Egg albumin	150.0 cc.
3. Na_2CO_3	3.6 g.
4. Nutrient agar (3.0%)	700.0 cc.

Preparation:

(1) Mix equal parts of 1 and 2 and add 3. (May be filtered thru thin layer of cotton to remove any thick parts of egg.)
(2) Steam (1) in Arnold sterilizer for 20 minutes.
(3) Prepare 3.0% nutrient agar neutral to litmus.
(4) Mix (2) and (3) while (3) is boiling hot.
(5) Pour medium thick plates, allow them to stand open for 20 to 30 minutes.

Sterilization: Method not given.

Use: Isolation of cholera vibrio. Authors reported that by transmitted light cholera colonies appeared deep in the agar with hazy appearance. Medium inferior to a whole egg medium.

Variants: Sacquépée (Bezançon) gave the following method of preparation:

(1) Add 200.0 cc. of sterile water to the whites of two eggs.
(2) Heat to 50°C.
(3) Make alkaline by adding 0.5 cc. of 10.0% soda solution for each 100.0 cc. of egg solution.
(4) Sterilize in the autoclave.
(5) Prepare 3.0% agar neutral to litmus.
(6) Add 9.0 cc. of 10.0% soda solution to a liter of (5).
(7) Add 200.0 cc. of (4) to 1000.0 cc. of (6).
(8) Sterilize in the autoclave at 115°C.

References: Krumwiede, Pratt and Grund (1912 p. 137), Bezançon (1920 p. 121).

1955. Oberstadt's Egg Albumin Agar

Same as medium 952 but the bouillon is solidified by the addition of agar.

1956. Besredka and Jupille's Egg Agar (Besson)

Constituents:

1. Nutrient agar	
2. Bouillon	500.0 cc.
3. Egg albumin (10.0% solution)	400.0 cc.
4. Egg yolk (10.0% solution)	100.0 cc.

Preparation:

(1) Mix 5 parts bouillon with 4 parts of a 10.0% solution of egg white in sterile water and one part of a 10.0% egg yolk emulsion in sterile water. If the eggs are not obtained under aseptic conditions, the solutions may be sterilized by heating at 55°C.
(2) Add 4.0 cc. of (1) to the surface of solidified agar and allow to stand for 24 hours.

Sterilization: Not specified.

Use: General culture medium.

Reference: Besson (1920 p. 55).

1957. Scales' Whole Egg Agar

Constituents:

1. Nutrient agar (1.5%)	70.0 cc.
2. Whole egg	20.0 cc.

Preparation:

(1) Strain fresh eggs thru four thicknesses of cheese cloth.
(2) Prepare 1.5% nutrient agar.
(3) Thoroly mix 20.0 cc. of (1) with 70.0 cc. of melted (2).

Sterilization: Sterilize at 12 pounds for 15 minutes.

Use: To study variation in morphology of *B. coli.*

Variants: The author added 1.0% glucose.

Reference: Scales (1921 p. 595).

1958. Stitt's Glycerol Egg Agar

Constituents:

1. Glycerol agar.
2. Egg.

Preparation:

(1) Take the yolk and white of one egg and mix thoroly with an equal amount of glycerol agar, at a temperature of 45 to 55°C.
(2) Tube.
(3) Inspissate in a rice cooker as for serum tubes.
(4) Since egg medium has a tendency to be dry it is well to add 1.0 cc. glycerin bouillon to each tube before sterilization.

Sterilization: Sterilize in the same manner as for blood serum.

Use: Cultivation of tubercle bacilli.

Reference: Stitt (1923 p. 38).

1959. Robertson's Alkaline Egg Agar (Park, Williams and Krumwiede)

Constituents:

1. Water	500.0 cc.
2. Egg	
3. NaOH (normal solution)	6.0 cc.
4. Nutrient agar	

Preparation:

(1) Mix the yolk of one egg, the whites of two eggs, 500.0 cc. of water and 6.0 cc. of N/1 NaOH.

(2) Mix one part (1) with five parts agar.

Sterilization: Not specified.

Use: Cultivation of cholera organisms.

Reference: Park, Williams and Krumwiede (1924 p. 126).

1960. Cantani jun's Sperm Agar

Constituents:

1. Nutrient agar.
2. Sperm.

Preparation:

(1) Remove the testicles from a bull under aseptic conditions.

(2) Extract the sperm by pressing the *Funiculus sperm aticus* between two fingers and remove the sperm, thus pressed out with a sterile platinum loop.

(3) Streak the sperm on sterile agar slants.

(4) Incubate to prove sterility.

Sterilization: Not specified.

Use: Cultivation of influenza bacilli, tubercle bacilli and others.

Variants: A similar medium was prepared as follows:

(1) Prepare nutrient agar.

(2) Free testicular material of its capsule.

(3) Wash the outer surface with alcohol and ether.

(4) When dry cut cross sections with a sterile knife.

(5) Remove some of the liquid from the cross sections by means of a strong platinum loop.

(6) Streak the liquid on sterile solidified agar slants.

(7) Incubate 10 hours to prove sterility.

References: Cantani jun (1897 p. 601).

1961. Pettersson's Brain Ascitic Fluid Agar

Constituents:

1. Nutrient agar.
2. Ascitic fluid.
3. Brain.

Preparation:

(1) Remove the brain under aseptic conditions from a dead new-born human foetus, and place in a sterile covered jar.

(2) Add sterile ascitic fluid to (1) and shake the mixture for an hour in a shaking machine.

(3) Place in the ice box and allow to stand for several hours.

(4) Mix the clear opalescent fluid with an equal amount of 3.0% agar.

Sterilization: Not specified.

Use: Cultivation of gonococci. The use of glucose agar increases the vigor of growth.

Reference: Pettersson (1920 p. 1385).

1962. Smith and Taylor's Fetus Agar

Constituents:

1. Nutrient agar.
2. Fetus tissue.

Preparation:

(1) Prepare nutrient agar.

(2) Slant. The tube should contain a small quantity of water of condensation or add a little bouillon.

(3) Add to each slant $\frac{1}{2}$ g. of tissue or an equivalent amount of stomach or other fluid or intestinal contents of the fetus.

(4) Seal the tubes with sealing wax.

Sterilization: Not specified.

Use: Isolation of *Vibrio fetus*.

Reference: Smith and Taylor (1919 p. 301).

1963. Noguchi's Ascitic Fluid Tissue Agar

Constituents:

1. Nutrient agar (2.0%)	200.0 cc.
2. Ascitic fluid	100.0 cc.
3. Tissue (rabbit testicle or kidney)	

Preparation:

(1) Prepare 2.0% nutrient agar.

(2) Adjust (1) to slightly alkaline.

(3) Place small pieces of fresh tissue (preferably rabbit testicle or kidney, but human placenta, sheep testicle or other sterile organs will suffice) into a tube measuring 2 by 20 cm.

(4) Mix 2 parts melted (2) with one part ascitic (or hydrocele) fluid and add 15.0 cc. to each tube of (3).

(5) After solidification add a layer of sterile paraffin to prevent evaporation.

(6) Incubate to test sterility.

Sterilization: Not specified.

Use: Isolation of *Treponema pallidum* Inoculate by forcing small bits of pallidum-containing tissue far into the butt of the tube. Other investigators cultivated a variety of organisms on similar media.

Variants:

(a) Zinsser, Hopkins and Gilbert prepared a similar medium as follows for the cultivation of large quantities of *Treponema pallidum.*

(1) Prepare serum agar.
(2) Melt (1) and pour to the height of about one inch into the bottom of a 200.0 cc. flask.
(3) Drop in sterile bits of tissue.
(4) Inoculate.
(5) When the agar has solidified, cover with a mixture of either salt solution and heated ascitic fluid, or slightly acid broth and ascitic fluid. Fill to the neck of the flask.
(6) Add a few bits of sterile tissue.
(7) Cover with liquid paraffin oil. Authors reported that large quantities of *Treponema pallidum* may be found within periods of from two to four weeks. It is not necessary to add the agar. The tissues may be heated in the autoclave.

(b) Flexner, Noguchi and Amoss cultivated organisms from poliomyelitic tissue on a medium prepared as follows:

(1) Prepare 1.0% nutrient agar and bouillon.
(2) Introduce a fragment of kidney into an Erlenmeyer or Florence flask of 100.0 cc. capacity. The inoculum is placed on the kidney.
(3) Mix equal volumes of agar and ascitic fluid. (Agar to be melted and cooled at 40°C.)
(4) Pour 15.0 cc. of (3) over the kidney (giving a solid layer 1 cm. deep).
(5) When solidified add 50.0 cc. of an equal mixture of sterile ascitic fluid and bouillon.
(6) Cover with a layer of sterile paraffin to yield a layer about 1 cm. deep.
(7) Incubate at 37°C.

(c) Rosenow and Towne cultivated polemorphic streptococci (causing poliomyelitis) on a medium prepared as follows:

(1) Prepare 1.5% nutrient agar.
(2) Mix two parts (1) with one part ascitic fluid.
(3) Method of sterilization not given.
(4) Distribute in test tubes of 0.8 cc. in diameter to a depth of 13 centimeters.
(5) Add to each tube a piece of fresh sterile rabbit kidney.
(6) Add a layer of sterile mineral oil.

(d) Loewe and Strauss cultivated organisms causing epidemic encephalitis on a medium prepared as follows:

(1) Mix one part sterile 2.0% nutrient agar with four or five parts ascitic fluid. Ascitic fluid should be sterile, contain no bile or fibrin, and have a high specific gravity.
(2) Place kidney into tube and cover with (1).
(3) Inoculate.
(4) Pour on or cover (2) with autoclaved petrolatum and incubate.

(e) Stitt cultivated treponemata on a medium prepared as follows:

(1) Prepare a 2.0% nutrient agar.
(2) Adjust (1) so that it is slightly alkaline.
(3) Mix two parts (2) with one part ascitic or hydrocele fluid.
(4) Fill 2 by 20 cm. test tubes by the addition of 15.0 cc. of (3).
(5) Place a fragment of fresh sterile rabbit kidney or testicle in each tube. The tissue should be in the bottom of the tube.
(6) After solidification add sterile paraffin oil so that it covers the solid medium to a depth of 3 cm.
(7) Inoculate in the bottom of the tube by means of a capillary pipette.

References: Noguchi (1912 p. 91), Flexner, Noguchi and Amoss (1915 p. 92), Zinsser, Hopkins and Gilbert (1915 p. 215), Rosenow and Towne (1917 p. 177), Loewe and Strauss (1920 p. 253), Stitt (1923 p. 53).

1964. Gőzony's Kidney Agar

Constituents:

1. Water	500.0 cc.
2. Bouillon	500.0 cc.
3. Peptone	20.0 g.

4. Agar	5.0 g.
5. Kidney (dog)	
6. Na_2CO_3(10%)	5.0 cc.

Preparation:

(1) Prepare bouillon.

(2) Mix equal parts bouillon and water.

(3) Dissolve 2.0% peptone and 0.5% agar in (2).

(4) Add 5.0 cc. of a 10.0% Na_2CO_3 solution to give a slightly alkaline reaction.

(5) Distribute in tubes.

(6) Cool sterile (5) to 45°C. and to each tube add a small piece of sterile dog kidney under aseptic conditions.

Sterilization: Sterilize (5) in the autoclave.

Use: Cultivation of flagellates. Author reported that the flagellates grown in this medium were larger than those grown in blood culture.

Reference: Gózony (1920 p. 566).

1965. Duval's Trypsinized Tissue Agar

Constituents:

1. Nutrient agar.
2. Tissue.
3. Trypsin (1.0% solution).

Preparation:

(1) Pour nutrient agar in sterile Petri dishes and allow to solidify.

(2) Cut an excised leprous nodule into thin slices, two to four millimeters in breadth and 0.5 to 1.0 mm. in thickness and distribute over the surface of the plate.

(3) Bathe the medium with a 1.0% trypsin solution, taking care not to submerge the pieces of leprous tissue. Add sufficient fluid to moisten thoroly the surface of the medium.

(4) Incubate in a moist chamber at 37°C. for a week to 10 days. Remove the plates from time to time and add more trypsin as evaporation necessitates.

Sterilization: Not specified.

Use: Cultivation of *B. leprae*. The author reported that the colonies were grayish white, but after several days they assumed a distinct orange yellow tint.

Reference: Duval (1911 p. 369).

1966. Thoinot and Masselin's Gelatin Agar

Constituents:

1. Bouillon	1000.0 cc.
2. Gelatin	100.0 g.
3. Agar (0.5%)	5.0 g.

Preparation:

(1) Dissolve 100.0 g. of gelatin in a liter of ordinary bouillon.

(2) Make alkaline.

(3) Add 0.5% agar and dissolve by boiling.

(4) Cool to 55°C. and add the white of an egg. Mix well.

(5) Boil 20 minutes at 115°C.

(6) Distribute as desired.

Sterilization: Sterilize for 20 minutes at 113 to 114°C.

Use: General culture medium.

Variants: Besson (Tanner) prepared the medium as indicated:

(1) Dissolve 80.0 g. of gelatin in bouillon.

(2) Neutralize.

(3) Dissolve 5.0 g. of agar in (2). 50.0 g. of gelatin and 8.0 g. agar may be used instead of 80.0 g. of gelatin and 5.0 g. of agar.

(4) Sterilize. Do not heat over 115°C.

References: Thoinot and Masselin (1902 p. 36), Tanner (1919 p. 56).

1967. Fremlin's Phosphate Gelatin Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. Potassium phosphate	10.0 g.
4. Bouillon gelatin	50.0 g.

Preparation:

(1) Exact composition or method of preparation of bouillon gelatin not given.

(2) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: To study nitrification by nitroso bacteria. Author reported that sub-cultures from this medium to other plates did not show nitrification, proving that this is not a suitable medium for continued activity.

Reference: Fremlin (1914 p. 154).

1968. Vierling's Fat Agar

Constituents:

1. Nutrient agar	100.0 cc.
2. Lanolin	10.0 g.

Preparation:

(1) Prepare nutrient agar.

(2) Prepare an emulsion of 1.0 g. lanolin in 10.0 cc. nutrient agar.

Sterilization: Not specified.

Use: To study lipase production by Mycobacteria. Author reported that good growth occurred. Crystals of salts of fatty acids could not be demonstrated.

Variants: The author gave the following variants:

(a) Used beef fat instead of lanolin.

(b) Vierling reported that on the following medium the fat was saponified causing the agar to become turbid and the crystalline salts of fatty acids to form. The layer of fat was not destroyed in any place.

  (1) Pour a thin layer of beef fat into Petri dishes.

  (2) When the fat has solidified pour a layer of glucose agar that has been cooled to 45°C. over the layer of fat.

Reference: Vierling (1920 p. 205).

1969. Abe's Meat Water Infusion Agar

Constituents:

1.	Water	1000.0 cc.
2.	Nutrient agar (2.0%)	
3.	Beef	500.0 g.

Preparation:

(1) Chop 500.0 g. of fat free beef in a meat chopping machine.

(2) Mix (1) and 1000.0 cc. of water, and allow to stand for 18 to 24 hours in an ice box.

(3) Filter the liquid thru filter paper and then thru a Chamberland filter.

(4) Distribute this sterile reddish liquid into sterile test tubes.

(5) Melt tubes of 2.0% sterile nutrient agar.

(6) Heat (4) to 45–50°C.

(7) To each 5.0 cc. of (5) add 2.0 cc. of (6).

(8) Mix thoroly.

(9) Pour into sterile Petri dishes.

Sterilization: Method of sterilization of nutrient agar not given. The infusion is sterilized by filtration, see step (3) above.

Use: Cultivation of gonococci. Author reported that gonococci colonies appeared after 18 hours at 37°C. as light gray small typical colonies. Kligler used a similar medium to study effect of extracts on growth of pathogenic organisms.

Variants:

(a) Kligler prepared media as given below to study effect of extracts on growth of pathogenic organisms:

  (1) Prepare nutrient agar.

  (2) Mascerate equal weights of fresh lean beef heart.

  (3) Take up each portion of (2) with 9 volumes of saline solution.

  (4) Treat each of these suspensions in one of the following ways:

    (a) Keep in the ice box over night and steam in an Arnold sterilizer for one hour.

    (b) Keep in the ice box over night and filter thru a Berkefeld candle.

    (c) Keep at 55°C. over night and heat in an Arnold sterilizer for one hour.

    (d) Keep at 55°C. over night and filter thru a Berkefeld candle.

    (e) Extract by boiling one hour and then sterilize in an Arnold sterilizer for one hour.

    (f) Extract by boiling for one hour.

  (5) Take 1.0 cc. of one of the extracts obtained and add to 5.0 cc. of sterile nutrient agar.

  Kligler reported that heat destroyed the growth stimulating material in the extract. Best growth in (4) (b), no stimulated effects in (4) (e) and (f).

(b) Kligler also used the following media to study the effects of growth accessory substances on pathogenic bacteria:

  (1) Prepare nutrient agar.

  (2) Obtain beef heart, rabbit tissues, rabbit organs, cat organs, cat tissues or mucosa of various organs as free from blood as possible under aseptic conditions.

  (3) Wash (2) with saline solution to remove all visible traces of blood.

  (4) Weigh and chop into small bits and suspend in 9 times the weight of saline solution.

  (5) Shake thoroly and place in the ice box over night.

  (6) Centrifuge and filter thru a Berkefeld candle.

  (7) Test sterility before using.

  (8) Tube (1) in 5.0 cc. lots.

  (9) Add amounts of (7) to each tube of (8) varying from 0.01 cc. to 1.0 cc.

References: Abe (1907 p. 707), Kligler (1919 pp. 32, 43).

1970. Esch's Hydrolyzed Meat Agar (Köhlisch and Otto)

Constituents:

1. Nutrient agar	
2. Beef	500.0 g.
3. NaOH (normal)	250.0 cc.

Preparation:

(1) Dissolve 500.0 g. of beef in 250.0 cc. of N/1 NaOH solution in an aluminum kettle by heating. Stir constantly. Solution is complete after from about 10 to 15 minutes.
(2) When cool filter thru a towel.
(3) Mix 3 parts sterile (2) with 7 parts neutral nutrient agar.
(4) Pour into plates.
(5) Dry at 60° for 30 minutes.
(6) Allow the plates to stand open at room temperature for 24 hours.

Sterilization: Sterilize in streaming steam for one hour. Method of sterilization of agar not given.

Use: Elective medium for cholera vibrio.

Variants:

(a) The author used pike instead of beef.
(b) Stitt prepared the medium as follows:
  (1) Treat 500.0 g. chopped beef with 250.0 cc. of normal NaOH until disintegrated.
  (2) Filter thru cloth.
  (3) Sterilize (method not given).
  (4) Add about one part of (3) to about 2.5 or 2 parts of nutrient agar.
  (5) Dry the plates.

References: Köhlisch and Otto (1915 p. 435), Stitt (1923 p. 50).

1971. Wellman's Placenta Infusion Agar

Add Wellman's Placenta Infusion (see medium 1377) to 2.0% nutrient agar.

1972. Orcutt and Howe's Fat Blood Agar

Same as medium 960 but solidified by the addition of agar.

1973. Dieudonne's Alkaline Blood Agar

Constituents:

1. Nutrient agar	700.0 cc.
2. Blood, defibrinated beef	150.0 cc.
3. KOH N/1	150.0 cc.

Preparation:

(1) Prepare nutrient agar and neutralize to litmus.
(2) To 150.0 cc. of defibrinated beef blood add an equal part of N/1 KOH.
(3) To 7 parts melted (1) add 3 parts (2), mix well and pour into plates.
(4) Dry the plates for several days at 37°C. or for 4 minutes at 60°C.

Sterilization: Sterilization of agar not specified. The laked blood alkali mixture may be sterilized in the autoclave.

Use: Enrichment medium for cholera vibrio. Author reported that cholera vibrio grew very luxuriantly on this medium. *B. coli* was inhibited.

Variants:

(a) Sineff and Drosdowitsch gave the following method of preparation:
  (1) Collect beef blood in a sterile enamel container and defibrinate it with a sterile egg beater.
  (2) Strain thru a fine tin sieve.
  (3) Mix equal parts of the filtrate from (2) and a normal solution (5.61%) of KOH.
  (4) Sterilize for one-half hour.
  (5) Mix 7 parts sterile agar and 3 parts (4) (sterile alkaline defibrinated blood).
  (6) Pour into sterile Petri dishes.
  (7) Dry at 37°C. for 24 hours.
(b) Hofer and Hovorka prepared a similar medium as follows:
  (1) To 3.0% melted nutrient neutral agar add 4.0 cc. of defibrinated beef blood, and 16.0 cc. of N/1 KOH.
  (2) Boil.
  (3) Distribute into 10.0 cc. lots.
  (4) Prepare a 0.1% solution of crystal violet in distilled water.
  (5) To each 10.0 cc. of (3) add 0.5 cc. of (4).
  (6) Pour into Petri dishes.
  (7) Place the plates in the incubator for 24 hours with covers partly removed and then at room temperatures for 12 hours with covers on before use.
(c) Lentz prepared the medium as follows:
  (1) Mix fresh defibrinated beef blood with equal amount of N/1 alkali.
  (2) Heat 30 minutes.
  (3) Dry in Faust-Heim drying apparatus.

(4) Grind in mortar.
(5) Preserve in glass stoppered bottle.
(6) For use dissolve 3.0 g. powder in 30.0 cc. distilled water. Add to 70.0 cc. neutral agar.

(d) Roddy gave the following method of preparation:
(1) Obtain ox blood from the slaughter house.
(2) Collect the blood in small sterile bottles containing glass beads.
(3) Shake until the blood is defibrinated.
(4) Mix equal volumes of (3) and normal sodium hydrate solution.
(5) Sterilize (3) in a steam sterilizer for 15 minutes on each of 2 successive days.
(6) Add 1 part of (5) to 7 parts sterile nutrient agar.
(7) Tube.
(8) Slant.
(9) Do not have the cotton plugs tighter than necessary.

(e) Stitt used normal NaOH instead of KOH.

References: Dieudonne (1909 p. 108), Sineff and Drosdowitsch (1909 p. 429), Hofer and Hovorka (1913 p. 111), Lentz (1915 p. 425), Roddy (1917 p. 45), Ball (1919 p. 83), Klimmer (1923 p. 218), Stitt (1923 p. 50), Park, Williams and Krumwiede (1924 p. 130).

1974. Pilon's Alkaline Blood Agar

Constituents:
1. Nutrient agar (4.0%) .... 70.0 cc.
2. Blood, defibrinated .... 15.0 cc.
3. Na_2CO_3 (crystalline 12.0% solution)

Preparation:
(1) Mix defibrinated blood and a 12.0% crystalline Na_2CO_3 solution in equal parts.
(2) Prepare 4.0% nutrient agar.
(3) Neutralize (2).
(4) Add 3 parts non-sterilized to (1), 7 parts melted (3) and mix thoroly.
(5) Pour into Petri dishes and allow the plates to solidify.

Sterilization: If the plates are to be used at once sterilization is not necessary due to high alkali content. If the plates are to be kept several days, draw blood under aseptic conditions and use sterilized agar.

Use: Enrichment medium for cholera vibrio.

Variants:

(a) Klimmer prepared the medium as follows:
(1) Mix 150.0 cc. of fresh defibrinated beef blood, with 150.0 cc. of a 12.0% soda solution.
(2) Shake well.
(3) Allow to stand for 1 to 6 days.
(4) Steam for 60 to 90 minutes.
(5) Mix 30 parts (4) with 70 parts sterile neutral agar.

(b) Park, Williams and Krumwiede gave the following method of preparation:
(1) Mix equal parts defibrinated beef blood and 12.0% Crystalline soda solution.
(2) Steam in the Arnold for 30 minutes.
(3) Prepare 3.0% agar neutral to litmus (about pH 6.6).
(4) Mix 3 parts (2) with 7 parts (3). Sterilization not specified.
(5) Pour into Petri dishes (15.0 cc. in a 10 cm. dish).
(6) Allow to harden, uncovered but protected by paper.
(7) Plates can be used after drying 30 minutes.

References: Pilon (1911 p. 331), Klimmer (1923 p. 218), Park, Williams and Krumwiede (1924 p. 130).

1975. Fildes' Pepsinized Blood Agar

Same as 961 but using nutrient agar instead of bouillon.

1976. Carpano's Hemolysed Blood Agar

Constituents:
1. Nutrient agar (2.5%) .... 1000.0 cc.
2. Hemolysed defibrinated blood

Preparation:
(1) Prepare 2.5% nutrient agar (peptone broth with 2.5% agar).
(2) Neutralize to phenolphthalein.
(3) Adjust the reaction by the addition of 4.0% normal HCl.
(4) Add sterile, defibrinated blood naturally hemolysed (details of preparation not given in the abstract) to the solidified agar.

Sterilization: Not given.

Use: Cultivation of gonococci.

Reference: Carpano (1919 p. 599) from (1920 p. 176).

1977. Mandelbaum's Lactose Blood Agar

Constituents:

1. Nutrient agar.
2. Lactose.
3. Rosolic acid.
4. Blood, defibrinated human.

Preparation:

(1) To a tube containing 8–10.0 cc. nutrient agar is added 0.3 g. lactose.
(2) Heat to 100°C.
(3) Cool to 50°C.
(4) Add 0.3 cc. 1.0% alcoholic rosolic acid solution.
(5) Add 1.0 cc. defibrinated sterile human blood (Probably animal blood may be used).
(6) Mix and pour into Petri dishes.
(7) Stand 24 hours, or dry ½ hour at 37°C.

Sterilization: Not specified.

Use: Isolation of colon typhoid group.

Reference: Mandelbaum (1912 p. 306).

1978. Thompson's Glucose Plasma Agar

Constituents:

1. Nutrient agar (2.5%)	1000.0 cc.
2. NaCl	9.0 g.
3. $CaCl_2$	0.25 g.
4. KCl	0.42 g.
5. Glucose (2.5%)	25.0 g.
6. Plasma (human)	

Preparation:

(1) Prepare nutrient agar (2.5%) in the ordinary way using 1.0% Witte peptone (Omit the NaCl).
(2) Adjust to +6 acid.
(3) Add 2, 3, 4 and 5 to (1).
(4) Tube in 4.0 cc. lots.
(5) Melt sterile (4) in boiling water and cool to 50°C. Then add 1.0 cc. of human plasma to each tube. Mix and slant.

Sterilization: Method of sterilization of (4) not given.

Use: Cultivation of gonococci.

Reference: Thompson (1917 p. 869).

1979. Avery's Oleate Blood Agar (Stitt)

Constituents

1. Nutrient agar	95.0 cc.
2. Sodium oleate (2.0% soln.)	5.0 cc.
3. Blood, defibrinated or citrated	

Preparation:

1 Prepare nutrient agar.
(2) Adjust (1) to pH = 7.2 to 7.5.
(3) To 95.0 cc. of (2), add 5.0 cc. of a 2.2% solution of neutral sodium oleate.
(4) Add defibrinated or citrated blood to (3) while it is still hot (temperature or amount of blood not specified).

Sterilization: Not specified.

Use: Cultivation of Pfeiffer's bacilli.

Reference: Stitt (1923 p. 43).

1980. Esch's Ascitic Fluid Blood Agar

Constituents:

1. Nutrient agar	60.0 cc.
2. Blood, defibrinated sheep	20.0 cc.
3. Ascitic fluid	10.0 cc.
4. Maltose	1.0 g.
5. Bouillon	3.0 cc.

Preparation:

(1) Prepare nutrient agar containing 1.0% Witte's peptone.
(2) Prepare bouillon.
(3) Mix 60.0 cc. of (1) that has been cooled to 50°C. with 20.0 cc. sterile defibrinated sheep blood, 10.0 cc. ascitic fluid and 1.0 g. of maltose dissolved in 3.0 cc. of bouillon.
(4) Pour into sterile Petri dishes.

Sterilization: Not specified.

Use: Cultivation and isolation of meningococci. Author reported that typical colonies developed after 8 hours.

References: Esch (1909 p. 153), Klimmer (1923 p. 225).

1981. Liston's Trypsinized Casein Blood Agar

Constituents:

1. Distilled water	100.0 cc.
2. Blood	20.0 cc.
3. Trypsinized casein	100.0 cc.
4. Agar (3.0%)	400.0 cc.

Preparation:

(1) A 200.0 cc. capacity flask, containing a few glass beads and 100.0 cc. distilled water, is sterilized and kept ready.
(2) 20.0 cc. of human (or rabbit) blood, removed with due precautions to avoid contamination, are added to this sterile distilled water. The flask is then at once vigorously shaken to prevent the formation of large fibrin masses and the blood soon lakes.
(3) 5.0 cc. of an alcoholic pancreatic extract are then added to the flask,

this extract being prepared locally from goat's pancreas, according to the method recommended by Cole and Onslow (Lancet (1916, see medium 1130.) Pig's pancreas was not used for various reasons.

(4) Next 5.0 cc. of the enterokinase solution are added with a view to hasten the trypsinization process. This enterokinase solution is a very dilute watery extract of the duodenal mucous membrane of goats and sheep which has been macerated in chloroform water.

(5) Lastly, 1.5 cc. of pure chloroform are added to the flask as a preservative and it is plugged with an India rubber cork to prevent the evaporation of the chloroform.

(6) The flask is then placed in the incubator at 37°C., after having shaken it well in order to mix the contents thoroly.

(7) On the second day the flask will be found to contain a well-settled sediment with a clear supernatant reddish fluid. The flask is shaken again to resuspend the sediment and put back into the incubator. The shaking is repeated on the third day, but after that the flask is allowed to stand undisturbed in the incubator until the eighth day.

(8) On the eighth day the flask is carefully removed so as not to disturb the sediment and the clear supernatant fluid is removed with aseptic precautions. In case the sediment has not settled firmly at the bottom, as much of the clear fluid should be removed as possible and the remainder filtered thru sterile filter paper, making arrangements to maintain sterility.

(9) Broth prepared from casein after the method recommended by Cole and Onslow (see medium 1130) is kept ready on hand.

(10) Tubes, each containing about 4.0 cc. of 3.0% agar, are kept ready sterilized. The agar is melted and cooled to a temperature of about 45°C.

(11) One cc. of the stock broth and 1.0 cc. of the blood fluid are well mixed; the tube is sloped, and the agar allowed to cool.

(12) Agar slopes thus prepared are transparent and have a slightly more golden color than ordinary agar.

Sterilization: Final sterilization not specified.

Use: Cultivation of *B. influenzae* (Pfeiffer). The author reported that agar slopes containing the digested blood gave almost as good results as those containing in addition casein digest (stock broth of Cole and Onslow). Many involution forms, however, were developed on this simpler medium, but the addition of sodium phosphate eliminated these. A culture of *B. influenzae* inoculated on this medium showed a perceptible growth after six hours incubation at 37°C., and after 24 hours incubation a luxuriant growth was obtained. This growth had a characteristic translucency. Individual isolated colonies on this medium measured from 1.0 mm. to 2.0 mm. in 24 hours.

Reference: Liston (1918–19 p. 419).

1982. Bernstein's Basal Blood Agar

Constituents:

1. Nutrient agar	150.0 cc.
2. Blood, beef	10.0 cc.

Preparation:

(1) Prepare nutrient agar with 1.0% of one of the added nutrients.

(2) Draw 400.0 cc. of beef blood directly into sterile Erlenmeyer flasks of 500.0 cc. capacity containing 35.0 cc. of a 1.0% solution of ammonium oxalate in distilled water.

(3) Shake for one or two minutes.

(4) Add 0.5 cc. of 40 volume formalin and allow the flask to stand undisturbed for an hour.

(5) Distribute the blood into sterile Erlenmeyer flasks and dilute with twice its volume of sterile (0.9%) saline solution.

(6) Allow the blood to stand for 24 hours to 48 hours at room temperatures before use.

(7) Seal the flasks and keep on ice until ready for use.

(8) To 15.0 cc. of sterile melted (1), add 1.0 cc. of (6).

(9) Pour into sterile plates.

Sterilization: Method not given.

Use: To study fermentation and hemolysis. Author reported that typhoid colonies did not produce hemolysis on lactose plates. Colon colonies did produce hemolysis on lactose plates. On raffinose, typhoid colonies showed umbilicated colonies with lines radiating from the center. Colon colonies did not show these lines. On maltose typhi were deeply pigmented, almost black; colon colonies were white. On dextrin typhoid colonies caused precipitation of the medium and were black. Colon colonies caused hemolysis and were white.

Variants: The author added 1.0% of any desired carbohydrate to nutrient agar.

Reference: Bernstein (1909 p. 2).

1983. Wordley's Oxalated Blood Agar

Constituents:

1. Nutrient agar............... 150.0 cc.
2. Blood (human oxalated)..... 10.0 cc.

Preparation: (1) Prepare nutrient agar.

(2) Melt agar and pour into sterile Petri dishes in 15.0 cc. lots.

(3) Add 1.0 cc. of human oxalated blood to each Petri dish.

Sterilization: Not specified.

Use: To study hemolysis by streptococci.

Reference: Wordley's (1921 p. 66).

1984. Wilson and Darling's Laked Blood Agar

Constituents:

1. Water...................... 10.0 cc.
2. Nutrient agar
3. Blood, sheep............... 1000.0 cc.
4. Sodium citrate............. 10.0 g.

Preparation:

(1) Dissolve 10.0 g. of sodium citrate in 10.0 g. of water. Place this in a bottle with volume graduation marks.

(2) Take (1) to the slaughter house and when a sheep is slaughtered, after the first gush of blood, collect the blood in the bottle until a liter of fluid has been obtained.

(3) Shake the bottle vigorously during the process.

(4) Add 1.25 cc. of formalin to the liter of citrated blood and mix thoroly.

(5) Transfer the blood with aseptic precautions to a ground glass stoppered bottle and add 30.0 cc. of methylated ether.

(6) Stopper firmly and shake well.

(7) Incubate over night at 37°C.

(8) When required for use, 3.0 cc. of (7) are pipetted to 100.0 cc. of melted and cooled to 50°C. nutrient agar.

(9) Keep the blood agar at 50°C. for one hour before pouring into plates, if to be used at once. Or pour into plates and incubate at 37°C. over night. This drives off the ether, and if the plates are incubated over night, tests sterility.

Sterilization: Not specified.

Use: Isolation and preservation of meningococci. Also used for the cultivation of *B. influenzae* and anaerobes.

Reference: Wilson and Darling (1918 p. 105).

1985. Wilson and Darling's Lactose Blood Agar

Constituents:

1. Distilled water........ 10.0 cc.
2. Nutrient agar
3. Blood, sheep...... .. 1000.0 cc.
4. Sodium citrate........ 10.0 g.
5. Lactose............... 0.5 to 1.0%
6. Crystal violet (0.1% soln.)
7. Litmus solution (Kubel and Tiemann)

Preparation:

(1) Dissolve 10.0 g. of sodium citrate in 10.0 cc. of water. Place this in a bottle with volume graduation marks.

(2) Take (1) to the slaughter house and when a sheep is slaughtered, after the first gush of blood, collect the blood in the bottle until a liter of fluid has been obtained.

(3) Shake the bottle vigorously during the process.

(4) Add 1.25 cc. of formalin to the liter of citrated blood and mix thoroly.

(5) Transfer the blood with aseptic precautions to a ground glass stoppered bottle and add 30.0 cc. of methylated ether.

(6) Stopper firmly and shake well.

(7) Incubate over night at 37°C.
(8) To 100.0 cc. of sterile melted and cooled to 50°C. agar containing 0.5 to 1.0% lactose, add 3.0 cc. of (7), 1.0 cc. of a 0.1% crystal violet solution and 12.0 cc. of Kubel and Tiemann's litmus solution.
(9) Pour into plates.
(10) Incubate over night at 37° to drive off the ether.

Sterilization: Method of sterilization not given.

Use: Cultivation of colon-typhoid group.

Reference: Wilson and Darling (1918 p. 105).

1986. Stitt's Glycerol Blood Agar (Chocolate Agar)

Constituents:
1. Glycerol agar............... 100.0 cc.
2. Citrated blood

Preparation:
(1) Prepare glycerol agar.
(2) Melt (1) and while the tubes are at a temperature of about 90°C. add about 4.0 or 5.0% citrated blood (0.5 cc. to 10.0 cc. of agar in a tube).
(3) Mix thoroly avoiding bubbles.
(4) Pour in sterile plates.

Sterilization: Not specified.

Use: Cultivation of Pfeiffer's bacilli. Author reported that this medium was less satisfactory for isolation of Pfeiffer's bacillus than plain blood agar (due to its brown color).

Variants: Stitt employed nutrient agar instead of glycerol agar.

Reference: Stitt (1923 p. 43).

1987. Besson's Citrated Blood Agar

Constituents:
1. Nutrient agar.
2. Blood, citrated.

Preparation:
(1) Collect 400.0 cc. beef blood in a sterile flask containing citrate solution and 0.1 cc. of commercial formol.
(2) Shake thoroly.
(3) Store for several days at room temperature.
(4) Dilute (3) with three volumes of sterile physiological salt solution.
(5) Add one part (4) to 15 parts sterile agar.

Sterilization: Method of sterilization of agar not given.

Use: Cultivation of pneumococci, meningococci and gonococci.

Variants:
(a) Stitt prepared an opaque medium as follows:
  (1) Prepare 2.0 to 3.0% nutrient agar.
  (2) The reaction of (1) should be −0.3 to phenolphthalein.
  (3) To 100.0 cc. of (2) add 20.0 cc. of a mixture of equal parts blood and sodium citrate solution (1.0% in normal saline).
  (4) Pour into 10 plates.
(b) Stitt prepared a transparent medium as follows:
  (1) Prepare nutrient agar.
  (2) Mix equal parts 1.0% sodium citrate solution and blood.
  (3) Add 20 to 30 drops of (2) to 100.0 cc. of (1).
  (4) Pour into 10 plates.

References: Besson (1920 p. 31), Stitt (1923 p. 44).

1988. Bezançon, Griffon and LeSourd's Blood Agar

Constituents:
1. Nutrient agar.
2. Blood, rabbit.

Preparation:
(1) Prepare nutrient agar.
(2) Cool sterile melted agar tubes to 50°C.
(3) Add fresh rabbit blood, drawn under aseptic conditions. (Amount not given.)
(4) Mix the blood and agar.
(5) Slant and cool.

Sterilization: Method of sterilization of (2) not given.

Use: Cultivation of the Ducrey bacillus. A large variety of organisms were cultivated on the same or similar media by a number of investigators.

Variants:
(a) Thoinot and Masselin streaked agar slants or agar plates with whole or defibrinated blood, using a sterile pipette.
(b) Davis specified that one volume of freshly drawn rabbit blood be added to two volumes of 2.0% nutrient agar.
(c) Czaplewski specified the use of pigeon

blood and mixed with nutrient agar cooled to 45 to 60°C. The medium was flaky and of a brownish color and used for the cultivation of influenza bacilli.

(d) Müller cultivated fowl diphtheria bacilli on a medium prepared as follows:
  (1) Prepare nutrient agar.
  (2) Liquify (1) and place into sterile 400.0 cc. Erlenmeyer flasks in 250.0 cc. lots.
  (3) Allow about 25.0 cc. of blood to pass directly from the jugular vein of a goat into the melted agar or gelatin which has been cooled to about 45°C. (Dog and chicken blood gave good results also.)
  (4) Pour the blood medium into sterile Petri dishes.

(e) Savini and Savini-Castano cultivated influenza bacilli on a hematin agar prepared as follows:
  (1) Allow about 100.0 cc. of blood to coagulate.
  (2) Pour off the serum.
  (3) Cut the blood clot into fine pieces and place into a flask containing 100 to 150.0 cc. of N/1 NaOH or N/10 soda solution.
  (4) Boil for a long time over a free flame until all is well dissolved.
  (5) Filter and sterilize (method not given).
  (6) Prepare nutrient agar.
  (7) Melt sterile (6) and cool to about 50°C.
  (8) To each 10.0 cc. of (7) add 1.0 cc. of (5) and mix well without shaking violently.
  (9) Allow the agar to solidify over night in a cool place.

  Authors reported that influenza bacilli developed upon this medium when another organism grew side by side with it. *Staphylococcus aureus* gave best results for this purpose.

(f) Ito and Matsuzaki cultivated *Spirochaeta icterohaemorrhagiae* on the following medium:
  (1) Prepare nutrient agar.
  (2) Mix one part normal guinea pig blood or human blood with one or two parts melted agar at 50°C.
  (3) Add a drop of infected blood to the tubes before solidification and distribute by stirring the tubes.
  (4) Incubate at 15° to 37°C. Paraffin may be poured over the medium but growth may be obtained without it.

  Authors reported that the erythrocytes settled to the bottom of the agar before solidification, giving it an opaque and deeper color.

(g) Wollstein cultivated Pfeiffer's bacillus on the following media:
  (1) Prepare nutrient agar.
  (2) Adjust the agar to pH = 7.5.
  (3) Boil fresh rabbit blood for two minutes in a water bath.
  (4) Centrifuge.
  (5) Add 0.5 cc. of the resulting clear pale pink or yellow fluid to 20.0 cc. of the broth or two or three drops to 5.0 cc. of the melted agar.

(h) Levinthal (Klimmer) cultivated influenza bacilli on a medium prepared as follows. Klimmer reported that best results were obtained using a medium 24 hours old.
  (1) Melt 1000.0 cc. of neutral 2.0 or 3.0% agar and cool to 70°C.
  (2) Mix 50.0 cc. of fresh human or dog blood with (1). The blood may be defibrinated and stored on ice, if desired.
  (3) Heat (2) to boiling over a wire gauze until the agar begins to raise in the neck of the flask.
  (4) Repeat the boiling twice more.
  (5) Separate the agar from the coagulum by filtering thru sterile cotton or gauze filter. Do not heat again in steam.
  (6) Agar is alkaline to litmus.
  (7) Tube or pour in plates.

References: Bezançon, Griffon and Le-Sourd (1901 p. 3), Thoinot and Masselin (1902 p. 36), Davis (1903 p. 405), Czaplewski (1902 p. 668), Heinemann (1905 p. 128), Müller (1906 p. 519), Savini and Savini-Castano (1911 p. 493), Ito and Matsuzaki (1916 p. 558), Roddy (1917 p. 45), Wollstein (1919 p. 556), Ball (1919 p. 82), Bezançon (1920 p. 120), Kristensen (1922 p. 223), Klimmer (1923 pp. 225, 227), Stitt (1923 p. 44), Park, Williams and Krumwiede (1924 p. 125).

1989. Fleming's Brilliant Green Blood Agar

Constituents:

1.	Water	9.0 cc.
2.	Blood	1.0 cc.
3.	Nutrient agar	
4.	Brilliant Green	1 to 500,000

Preparation:

(1) Boil 1.0 cc. of blood with 9.0 cc. water. (Time not specified.)

(2) Add some of the clear supernatant liquid of (1) to nutrient agar. (Amount of liquid not specified).

(3) Add brilliant green in the ratio of 1 to 500,000.

Sterilization: Not specified.

Use: Isolation of *B. influenzae.* Author reported that pneumococci, staphylococci and streptococci were inhibited. *B. influenzae* apparently was not inhibited.

Reference: Fleming (1919 p. 139).

1990. Hachla and Holobut's Alkaline Blood Agar

Constituents:

1.	Nutrient agar	700.0 cc.
2.	Blood, beef	200.0 cc.
3.	KOH N/1	100.0 to 150.0 cc.

Preparation:

(1) Prepare neutral nutrient agar.

(2) Mix 200.0 cc. of beef blood with 100.0 cc. or 150.0 cc. of normal KOH.

(3) Steam (2) for 30 minutes.

(4) Mix 7 parts liquid (1) with 3 parts (3).

(5) Pour into sterile plates.

(6) Dry the plates for 24 hours at 37°C. and then for 24 more hours at room temperature.

Sterilization: Method not given.

Use: Enrichment medium for cholera vibrio. Authors reported that better results were obtained using 1 part blood to 0.75 normal KOH solution. Hog and horse blood gave as good results as did beef blood.

Reference: Hachla and Holobut (1909 p. 299).

1991. Matthews' Trypsinized Blood Agar

Constituents:

1.	Bouillon	4.75 cc.
2.	Trypsin (Allen and Hanbury's)	0.25 cc.
3.	Blood	1.0 cc.
4.	Nutrient agar	30.0 cc.

Preparation:

(1) Add 0.25 cc. of Allen and Hanbury's trypsin compound to a series of tubes containing 4.75 cc. of sterile bouillon.

(2) Incubate for 24 hours.

(3) Discard tubes showing contamination.

(4) Add 1.0 cc. of blood from a vein puncture to each tube.

(5) Incubate the mixture for 3 or 4 days.

(6) Mix 5.0 cc. of the finished product with about 30.0 cc. of nutrient agar, as in the preparation of ordinary blood agar.

Sterilization: Not specified.

Use: Isolation of influenza bacillus. Author reported that medium may be prepared in larger quantities. This amount is sufficient for a diagnosis, however. Do not allow the trypsin to act longer than one week. Citrated blood may be used. Pneumococci, streptococci and other gram positive organisms were inhibited. Staphylococci, however, were favored. Gram negative cocci of the catarrhalis group and bacilli of the coli form group were not inhibited, but were not favored as were the influenza.

Reference: Matthews (1918 p. 104).

1992. Bieling's Glucose Blood Agar

Constituents:

1.	Nutrient agar	
2.	Agar	150.0 cc.
3.	Blood, horse	50.0 cc.
4.	Glucose 2.0%	3.0 g.

Preparation:

(1) Mix one part horse blood with two parts water.

(2) Prepare nutrient agar.

(3) Add 2.0% glucose to (2).

(4) Melt the glucose agar and cool to 60°C.

(5) Mix equal parts (1) and (4).

Sterilization: Not specified.

Use: Differentiation of streptococci and pneumococci. Author reported that hemolytic streptococci did not change the color of the medium. Colonies flat, about 2 mm. in diameter, with a central point and a border. Colonies could easily be picked from the agar with a platinum loop after 24 to 48 hours. *Streptococcus mitior* colonies were about the same size, dark brown or brownish violet, dry and hard to remove from the agar. Pneumococci colonies were larger than streptococci, about 5 mm. in diameter, colonies were dark brown or brownish, forming a smooth, coherent mucilaginous membrane. A light yellow decolorization of the medium took place when pneumococci growth was luxuriant. If agar and blood were mixed at 50°C. a clear ruby red transparent medium was obtained. Heating above this temperature gave a darker medium. When mixed at 60°C. the medium was not transparent in thick layers.

References: Bieling (1921 p. 262), Klimmer (1923 p. 226).

1993. Mandelbaum and Heinemann's Glycerol Blood Agar (Kolle and Wassermann

Constituents:
1. Glycerol agar.
2. Blood human.

Preparation: (1) Streak glycerol agar with human blood.

Use: Cultivation of diphtheria bacilli. Author reported that diphtheria colonies were light with yellowish brown ring. Pseudo diphtheria colonies red. Similar media were used to cultivate a large number of organisms by a number of investigators.

Variants:

(a) Sick prepared a medium as follows for clinical purposes:
  (1) Draw blood under aseptic conditions directly from a vein into a sterile Erlenmeyer flask containing glass beads, using a sterile needle and sterile tube.
  (2) Shake the flask to defibrinate the blood.
  (3) Prepare 3.0% agar containing 2.0 to 3.0% glycerol.
  (4) Mix one part (2) with 4 or 5 parts sterile (3), cooled to 50°C.
  (5) Distribute into sterile test tubes.

(b) Besson (Tanner) gave the following method of preparation:
  (1) Melt tubes of glycerol agar and cool to 40°C.
  (2) Add 1.0 cc. of blood from a rabbit's artery.
  (3) Mix the tubes without shaking.
  (4) Cool in a slanting position.

(c) Bezançon and Griffon (Besson) gave the following method of preparation:
  (1) Add 5.0% glycerol to nutrient agar.
  (2) Liquify sterile (1) and cool to 45°C.
  (3) Add about 1.0 cc. of fresh rabbit blood. (A 1.0% solution of commercial hemoglobin may replace the blood.)
  (4) Mix well without shaking.
  (5) Slant and cool.

(d) Cantani (Besson) prepared a medium as follows:
  (1) Mix equal parts of sterile blood and sterile glycerol.
  (2) Allow to stand several hours.
  (3) Add 0.5 to 0.75 cc. of (2) to tubes of liquified sterile agar.

(e) Stitt cultivated pneumococci, streptococci, gonococci and meningococci on a medium prepared by smearing the surface of a glycerol agar slant with a platinum loop of blood obtained from the lobe of a well cleaned ear.

References: Kolle and Wasserman (1912 p. 414), Sick (1912 p. 111), Tanner (1919 p. 69), Besson (1920 pp. 53, 54), Stitt (1923 p. 47).

1994. Wasserman's Nutrose Blood Agar (Abel)

Constituents:

1.	Water	30.0 to 40.0 cc.
2.	Blood (hog)	15.0 cc.
3.	Glycerol	2.0 or 3.0 cc.
4.	Nutrose	0.8 g.
5.	Agar (2.0% peptone)	

Preparation:
(1) Mix 15.0 cc. of hog blood, 30.0 to 40.0 cc. of water, 2.0 to 3.0 cc. of glycerol and 0.8 g. nutrose.
(2) Shake the mixture constantly and boil for 15 minutes.
(3) Repeat the boiling and shake on the following day for 15 minutes.
(4) When desired for use heat to 50 to 60°C. and mix with an equal quantity of sterile 2.0% peptone agar.
(5) Pour into plates.

Sterilization: Not specified.

Use: Cultivation of gonococci.

Reference: Abel (1912 p. 162).

1995. Savini and Savini-Castano's Bacteria Blood Agar

Same as medium 977 but use nutrient agar instead of bouillon.

1996. Bieling's Optochin Hydrochloride Blood Agar

Constituents:

1. Distilled water	100.0 cc.
2. Optochin hydrochloride	0.1 g.
3. Nutrient agar (3.0%)	
4. Blood, horse	60.0 cc.

Preparation:

(1) Dissolve 0.1 g. optochin hydrochloride in 10.0 cc. distilled water (should not be kept more than 3 days).
(2) Prepare 3.0% nutrient agar.
(3) Mix 1.0 cc. of (1) with 150.0 cc. of (2).
(4) Mix 60.0 cc. horse blood with 90.0 cc. distilled water.
(5) Heat (4) for one hour at 60°C.
(6) Melt (3) and cool to 60°C.
(7) Mix equal parts (6) and (5), both being at 60°C.

Sterilization: Not specified.

Use: Differentiation of streptococci and pneumococci. Author reported that pneumococci showed no growth. Hemolytic streptococci colonies flat, and did not change the color of the medium. May be easily removed from the medium. *Streptococcus mitior* colonies were dry and brown and hard to remove from the medium.

References: Bieling (1921 p. 264), Klimmer (1923 p. 226).

1997. Heim's Hemoglobin Agar

Constituents:

1. Distilled water	90.0 cc.
2. Hemoglobin	10.0 g.
3. KOH (10%)	10.0 cc.
4. Nutrient agar	

Preparation:

(1) Mix 1, 2 and 3.
(2) Mix 1.0 cc. sterile (1) with 7.0 cc. nutrient agar.
(3) Pour into plates or slant.

Sterilization: Sterilize (1) in streaming steam.

Use: Cultivation of pneumococci. Also used by other investigators for the cultivation of influenza bacilli.

Variants:

(a) Savini and Savini-Castano reported influenza bacilli developed only upon the following medium when other organisms were growing side by side with it. *Staphylococcus aureus* gave best results for this purpose.
  (1) Prepare a solution of 5.0% hemoglobin in N/10 NaOH or soda solution. Boil until the solution is complete.
  (2) Filter and sterilize (1). (Method of sterilization not given.)
  (3) Prepare nutrient agar.
  (4) Melt sterile (3) and cool to about 50°C.
  (5) To each 10.0 cc. of (4) add 1.0 cc. of (2) and mix well without shaking violently.
  (6) Allow the agar to solidify over night in a cool place.

(b) Thalhimer cultivated *Bac. influenzae* on the following medium. He reported that a slightly better medium was obtained if a few cc. of hydrogen peroxide were added before filtering. Growth was even more luxuriant than on ordinary blood agar.
  (1) Dissolve amorphous powdered hemoglobin (Merck & Co., and Eimer and Amend) in 100.0 cc. of water until a deep mahogany brown color is obtained. (About 10.0 g. hemoglobin.)
  (2) Filter thru a Reichel porcelain filter.
  (3) Add enough of (2) to sterile fluid agar to obtain the same intensity of color as ordinary blood agar.
  (4) Tube and slant.

References: Heim (1907 p. 1587), Savini and Savini-Castano (1911 p. 493), Thalhimer (1914 p. 189).

1998. Esch's Alkaline Hemoglobin Agar

Constituents:

1. Distilled water.
2. Hemoglobin (Merck's).
3. Neutral agar.

Preparation:

(1) Triturate hemoglobin.
(2) Add 5.0 g. hemoglobin to 15.0 cc. N/1 NaOH + 15.0 cc. distilled water.

(3) Mix sterile (2) with 85.0 cc. neutral agar.

Sterilization: Sterilize (2) in streaming steam.

Use: Isolation of cholera vibrio.

Variants:

(a) Costa gave the following method of preparation:

(1) Pulverize crystalline blood in a mortar.

(2) Add distilled water slowly to (1). This forms sort of a sticky mixture.

(3) Add 100.0 cc. of a normal KOH solution to (2).

(4) Heat in the autoclave for 30 minutes.

(5) Filter.

(6) Sterilize by heating in the autoclave at 100°C. for several hours.

(7) Mix 3 volumes of (6) with 7 volumes of sterile melted agar under aseptic conditions.

(8) Pour into sterile Petri dishes.

(b) Köhlisch and Otto prepared the medium as follows:

(1) Rub 15.0 g. of Merck's horse haemoglobin in a mortar.

(2) Mix 15.0 cc. of N/1 NaOH with (1), and add 15.0 cc. distilled water.

(3) Sterilize for one hour in the steamer.

(4) To 15.0 cc. of (3) add 850.0 cc. of neutral agar.

(5) Mix thoroly.

(6) Pour in plates.

(7) Dry the plates at room temperatures for one hour.

(c) Klimmer used the following method in preparing the medium:

(1) Dissolve 50.0 g. of Merck's hemoglobin in 300.0 cc. of half normal KOH by heating in a steamer for an hour.

(2) To hot (1) add 1700.0 cc. of melted hot neutral agar.

(3) Pour into plates.

References: Esch (1910 p. 559), Costa (1912 p. 846), Köhlisch and Otto (1915 p. 438), Klimmer (1923 p. 218).

1999. Kabeshima's Alkaline Hemoglobin Agar

Constituents:

1. Nutrient agar (3.0%) .... 80.0 cc.
2. Soda (18.0% solution) .... 10.0 cc.
3. NaCl (0.85% solution) .... 10.0 cc.
4. Hemoglobin extract (Pfeuffer) 3.0 g.

Preparation:

(1) Place 80.0 cc. of melted neutral nutrient 3.0% agar in an Erlenmeyer flask.

(2) Add 10.0 cc. of 18.0% soda solution and boil this mixture for about 10 minutes.

(3) Cool to 50°C.

(4) Dissolve 3.0 g. hemoglobin extract (Pfeuffer) in 10.0 cc. of 0.85% NaCl solution.

(5) Mix (4) and (3) thoroly.

(6) Pour into 7 Petri dishes and allow to stand with covers removed until solidification has taken place.

(7) Place the dishes in an incubator with cover removed for 20 to 30 minutes to remove the water of condensation.

Sterilization: Not specified.

Use: Selective medium for cholera vibrio. Author reported that medium was transparent and dark brown. Some cholera-like vibrio and other organisms were inhibited on this medium.

Variants: Klimmer prepared the medium in the following manner:

(1) Dissolve 3.5 g. of Pfeuffer's hemoglobin extract in 10.0 cc. of physiological salt solution.

(2) Add 10.0 cc. of a 5.5% solution of water free soda and 2.0 cc. of KOH solution (strength not given) to (1).

(3) Steam for 15 minutes.

(4) Cool to 50°C.

(5) Add (4) to 80.0 cc. of 3.0% melted nutrient agar, neutral to litmus and cooled to 80 or 90°C.

(6) Mix thoroly.

(7) Pour in plates.

(8) Dry thoroly in the incubator.

References: Kabeshima (1913 p. 203), Klimmer (1923 p. 220).

2000. Besson's Glycerol Hemoglobin Agar (Tanner)

Constituents:

1. Glycerol agar.
2. Hemoglobin.

Preparation:

(1) Melt tubes of glycerol agar and cool to 40°C.

(2) Add 1.0 cc. of a solution of hemoglobin

to each tube, (strength of hemoglobin solution not given).

(3) Mix the tubes without shaking.

(4) Cool in a slanting position.

Sterilization: Not specified.

Use: General culture medium.

Reference: Tanner (1919 p. 69).

2001. Kabeshima's Hemoglobin Extract Agar

Constituents:

1. Nutrient agar (3.0%).......	1000.0 cc.
2. Hemoglobin extract........	50.0 g.
3. Saline solution.............	1000.0 cc.

Preparation:

(1) Add 0.1 cc. of an 18.0% Na_2CO_3 solution to 1000.0 cc. of neutrient neutral agar.

(2) Boil 10 minutes.

(3) Cool to about 15°C. and add 1000.0 cc. of hemoglobin extract prepared by dissolving 50.0 g. of Shinoda's hemoglobin extract in 1000.0 cc. of 0.9% salt solution.

(4) Distribute in petri dishes and remove the bubbles by flaming with a Bunsen Burner.

(5) When cool remove the covers and place in the incubator for 30 minutes.

(6) Use when dry.

Sterilization: Not specified.

Use: Cultivation of cholera vibrio.

Reference: Kabeshima (1916 #225) taken from (1917 p. 395).

2002. Finger, Ghon and Schlagenhaufer's Dialyzed Serum Agar

Constituents:

1. Nutrient agar.
2. Blood Serum (Dialyzed).

Preparation:

(1) Place sterile human blood serum in sterile parchment sack.

(2) Suspend the blood serum in the sack in a sterile cylinder containing sterile water.

(3) Change the water every 48 hours and continue the dialysis until the water is colored by the albumin that dialyzes thru the parchment.

(4) Mix the dialyzed blood serum with agar in the same manner as in the preparation of blood serum agar.

Sterilization: Method not given.

Use: Cultivation of gonococci. Authors reported that growth was equally as good in non-dialyzed serum medium.

Reference: Finger, Ghon and Schlagenhaufer (1894 p. 14).

2003. Müller's Serum Agar

Constituents:

1. Nutrient agar.
2. Serum, goat.

Preparation:

(1) Prepare nutrient agar.

(2) Melt (1) and cool to about 60°C.

(3) Mix equal parts of (2) and goat serum that has been obtained under aseptic conditions and heated to 60°C.

(4) Pour into sterile plates or sterile tubes.

Sterilization: Method of sterilization of agar not given.

Use: Cultivation of fowl diphtheria bacilli. Similar media were used for the cultivation of various organisms by a number of investigators.

Variants:

(a) Mühlens and Hartmann cultivated *Spirochaeta dentium* and *Bacillus fusiformis* on a medium prepared as follows:

(1) Prepare nutrient agar with a slightly alkaline or neutral reaction.

(2) Boil half filled tubes of (1) for about 30 minutes in a water bath to free the agar of oxygen.

(3) Heat horse serum for about 30 minutes in a water bath at a temperature from 58 to 60°C.

(4) Cool (2) and (3) quickly to 45°C.

(5) Mix two parts of the agar with one part of the serum.

(6) Inoculate while the medium is still in a liquid state.

(7) Solidify by placing the tubes in cold water.

(b) Carrel mixed serum with one-fifth its volume of 2.0% agar and used the medium for the cultivation of tissue.

(c) Besson prepared the medium as follows:

(1) Melt tubes of sterile nutrient agar.

(2) Cool to 45° to 50°C.

(3) Add one-half or one-third the volume of sterile serum to each tube.
(4) Mix thoroly by rolling the tube between the palms of the hands.
(5) Slant or pour into a Petri dish.

(d) Dopter and Sacquépée gave the following method of preparation:
(1) Add 0.5 to 1.0 cc. of serum to an agar slant.
(2) Incline so that the entire surface is covered with the serum.
(3) Allow to stand for 12 to 24 hours.

(e) Klimmer mixed one part gelatin agar with one-half part serum at a temperature of 45°C.

References: Müller (1906 p. 520), Mühlens and Hartmann (1906 p. 86), Carrel (1912 p. 393), Besson (1920 p. 53), Dopter and Sacquépée (1921 p. 138), Klimmer (1923 p. 200), Kligler and Robertson (1922 p. 315).

2004. Joos' Alkaline Serum Agar (Klimmer)

Constituents:

1. Distilled water	150.0 cc.
2. Serum	300.0 cc.
3. NaOH (normal)	50.0 cc.
4. Bouillon	500.0 cc.
5. Agar	20.0 g.

Preparation:
(1) Heat 1, 2 and 3 for 2 to 3 hours in a water bath at 60 to 70°C.
(2) Steam for 30 to 45 minutes.
(3) Prepare bouillon using 2.0% peptone and 1.5% NaCl.
(4) Adjust the reaction of (3) to alkaline.
(5) Dissolve 20.0 g. agar in (4).
(6) Mix (5) and (2).
(7) Filter.

Sterilization: Method not given.

Use: General culture medium.

Variants: Klein (Klimmer) gave the following method of preparation for a medium for the cultivation of diphtheria bacilli.
(1) Mix 9 parts serum with 1 part normal NaOH and incubate at 37°C. for 2 days.
(2) Neutralize by the addition of HCl.
(3) Mix 1 part (2) with 4 parts nutrient agar.
(4) Sterilize at 105°C. for 1 hour.

Reference: Klimmer (1923 p. 222).

2005. Muhlen's and Hoffman's Glucose Serum Agar (Stitt)

Constituents:

1. Nutrient agar (3.0%)	100.0 cc.
2. Glucose (0.5%)	0.5 g.
3. Serum (Horse)	100.0 cc.

Preparation:
(1) Fill sterile test tubes one-third full with horse serum.
(2) Add an equal amount of a 3.0% agar containing 0.5% glucose which has been melted and cooled to 50°C. to sterile (1).
(3) Keep at 55° for two hours.

Sterilization: Sterilize (1) on 3 successive days at 55°C.

Use: Cultivation of treponemata. The medium was inoculated while still liquid and incubated under anaerobic conditions.

Reference: Stitt (1923 p. 54).

2006. Cantanis' Glycerol Serum Agar (Besson)

Constituents:
1. Nutrient agar.
2. Glycerol.
3. Serum.

Preparation:
(1) Mix equal parts of sterile serum and sterile glycerol.
(2) Allow to stand several hours.
(3) Add 0.5 to 0.75 of (2) to tubes of liquified sterile agar.

Sterilization: Method not given.

Use: General culture medium.

Reference: Besson (1913 p. 54).

2007. Kodama's Fuchsin Sulphite Serum Agar

Constituents:

1. Water	40.0 cc.
2. Nutrient agar (3.0%)	100.0 cc.
3. Fuchsin (saturated alcoholic solution)	0.5 cc.
4. Na_2SO_3 (10.0% solution)	2.5 cc.
5. Starch, potato	3.0 g.
6. Serum, beef	10.0 cc.

Preparation:
(1) Prepare nutrient 3.0% agar, neutral to litmus.
(2) Add 1.0 cc. 10.0% soda solution, 0.5 cc. of a saturated alcoholic fuchsin

solution, 2.5 cc. of a freshly prepared sodium sulfite solution and 3.0 g. of potato starch (Merck) stirred up in 5.0 cc. water, to (1) and steam in a steamer for 30 minutes.
(3) To a 50.0 cc. of beef serum diluted with 5 parts water, add 0.5 cc. of a 10.0% NaOH solution and boil for 30 minutes in the steamer.
(4) Mix two parts (2) with one part (1).
(5) Distribute into sterile test tubes.
(6) Pour sterile (5) into sterile Petri dishes, seeing that the starch is well distributed. Dry the plates before use.

Sterilization: Sterilize on three successive days for 30 minutes each day in the steamer.

Use: Elective medium for cholera vibrio. Author reported that after 3 to 9 hours the cholera colonies were red. There were two types, one a flat colony with irregular edges and the other, smaller, raised and definitely defined edge. *Bact. coli*, *Bact. dysenteriae* Shiga-Kruse, *Bact. dysenteriae* Flexner, anthrax bacillus, *Staphylococcus pyogenes aureus*, etc., all formed colorless colonies. The cholera-like vibrio all gave one of the two indicated types of red colonies. Wheat starch gave the same results but an hour later. Ordinary water soluble starch (Merck) gave only the small well defined edge type of colony. Medium prepared with dextrin did not give as good results as using potato starch.

Variants: Kodama used 3.0 g. dextrin instead of starch.

Reference: Kodama (1922 p. 433).

2008. Wasserman's Nutrose Serum Agar

Constituents:

1. Water	30.0 to 35.0 cc.
2. Serum, hog	15.0 cc.
3. Nutrose	0.8 to 0.9 g.
4. Nutrient agar	
5. Peptone	
6. Glycerol	

Preparation:
(1) Place 15.0 cc. of hog serum as free from hemoglobin as possible in an Erlenmeyer flask and dilute with 30 to 35.0 cc. water.
(2) Add 2 or 3.0 cc. of glycerol to (1).
(3) Add 0.8 or 0.9 g. nutrose to (2).
(4) Heat over a free flame to boiling, shaking constantly. The turbid fluid becomes clear.
(5) Liquefy 2.0% peptone agar and cool to about 50°C.
(6) Mix equal parts (5) and sterile (4).
(7) Pour into sterile Petri dishes.

Sterilization: Heat (4) until sterile. With fresh serum about 20 minutes is required. With old serum a longer time is necessary and it is often best to heat on several successive days. Sterilization of agar not given.

Use: Cultivation of gonococci.

Variants: Klimmer used alkaline nutrient agar.

References: Wasserman (1898 p. 300), Klimmer (1923 p. 200).

2009. Kligler's Nasal Secretion Serum Agar

Constituents:
1. Glucose agar.
2. Serum, sheep.
3. Nasal secretion.

Preparation:
(1) Prepare glucose agar.
(2) Obtain nasal secretions by blowing the nose into sterile gauze.
(3) Place into saline solution or alcohol and keep 3 days.
(4) Divide the saline mixture into two equal parts.
(5) Filter one of (4) thru a Berkefeld and autoclave the other part.
(6) Desiccate the alcoholic extract to dryness and then take up in saline solution.
(7) Distribute (1) in 10.0 cc. lots.
(8) Add 1.0 cc. sheep serum to each tube of (7), also 1.0 cc. or 0.5 cc. of one of the various extracts (6) and one of (5).
(9) Pour into plates.

Sterilization: Method of sterilization of glucose agar not given.

Use: Show influence of nasal secretion extract on growth of meningococci. Author reported that saline extracts in each case were superior to alcoholic extract. Filtered saline extract more effective than heated one.

Reference: Kligler (1919 p. 39).

2010. Meyer's Tuberculin Agar

Constituents:

1. 5.0% glycerol bouillon with heavy growth of tubercle bacilli	200.0 cc.
2. Glycerol bouillon (4.0 or 5.0%)	200.0 cc.
3. Agar (2.0%)	8.0 g.
4. Serum (10.0%)	40.0 cc.

Preparation:

(1) Tuberculin flasks containing about 250.0 cc. of a 5.0% glycerol peptone bouillon, with a heavy growth of human or bovine tubercle bacilli, about 6 to 8 weeks old are steamed in a Board of Health sterilizer for two hours.

(2) Cool and filter thru fine filter paper and centrifuge to eliminate every tubercle bacillus.

(3) Mix the clear liquid with an equal volume of the same 4.0 or 5.0% glycerol peptone bouillon and 2.0% agar is added and dissolved.

(4) Filter and correct the reaction to 1.5.

(5) Distribute in 9.0 cc. quantities in test tubes.

(6) Before being slanted 1.0 cc. (or 10.0%) of fresh sterile horse or cattle serum is added to each tube of sterile agar.

Sterilization: Sterilize (5) in the autoclave.

Use: Cultivation of *Bacillus para tuberculosis*.

Reference: Meyer (1913 p. 175).

2011. Douglas' Tellurite Trypsinized Serum Agar

Constituents:

1. Nutrient agar (2.0%)	1000.0 cc.
2. Potassium tellurite	
3. Serum	
4. Trypsin	

Preparation:

(1) Prepare nutrient (2.0%) agar. (Preferably a trypsin digest agar, but ordinary peptone agar gave almost identical results).

(2) Add 1.0% potassium tellurite to 100.0 cc. of water and dissolve as much as possible.

(3) Allow the insoluble portion of (2) to settle and pour off the clear supernatant layer.

(4) Rub up the deposit with 10.0% KOH solution added drop by drop to prevent excess of alkali.

(5) Add HCl to (4) until a precipitate begins to appear. Add this fraction to the clear supernatant fluid from (3).

(6) Add sufficient glycerinated commercial trypsin to serum (horse generally) to partially neutralize the antitryptic power. (Requires from 2.0 to 8.0 cc. of trypsin per 100.0 cc. depending on the strength of the trypsin, 2 to 4.0 cc. of Fairchild & Co. and Digestive Ferments Co. trypsin being sufficient and 5.0 to 8.0 cc. of Allen and Hanbury's trypsin).

(7) Filter (6) thru a porcelain candle of medium porosity and distribute in sterile flasks.

(8) Melt sterile (1) and add 4.0 cc. of (5) for each 100.0 cc. of agar.

(9) Cool to between 65° and 60°C. and add 15.0 cc. of trypsinized serum per 100.0 cc. agar.

(10) Mix thoroughly.

(11) Tube in sterile tubes.

(12) Slant.

(13) Incubate to test sterility.

Sterilization: Method of sterilization of (1) and (5) not given.

Use: Cultivation of *B. diphtheriae*.

Reference: Douglas (1922 p. 263).

2012. Czaplewski's Alkaline Serum Glucose Agar

Constituents:

1. Glucose agar	
2. Serum	300.0 cc.
3. NaOH (normal)	30.0 cc.

Preparation:

(1) Add 30.0 cc. normal NaOH to 300.0 cc. sheep serum.

(2) Sterilize (method not given).

(3) Allow to stand and pour off the clear supernatant liquid.

(4) Mix sterile (3) with sterile glucose agar (1.0, 2.0 or 3.0 cc. serum to 10.0 cc. agar).

Sterilization: Sterilize (3) in the autoclave. Method of sterilization of glucose agar not given.

Use: Cultivation of diphtheria bacilli.
Reference: Czaplewski (1920 p. 828).

2013. Klein's Alkaline Serum Agar

Constituents:

1. Nutrient agar	400.0 cc.
2. Serum	90.0 cc.
3. NaOH (15.0%)	10.0 cc.

Preparation:
(1) Mix 90 parts serum with 10 parts 15.0% NaOH.
(2) Incubate 2 days at 37°C.
(3) Neutralize by the addition of HCl.
(4) Mix one part (3) with four parts nutrient agar.

Sterilization: Heat (4) for 30 minutes at 105°C.
Use: Cultivation of diphtheria bacillus.
Reference: Klein (1920 p. 297).

2014. Frost, Charlton and Little's Milk Serum Agar

Constituents:

1. Distilled water	100.0 cc.
2. Nutrient agar	200.0 cc.
3. Milk	200.0 cc.
4. Serum	100.0 cc.

Preparation:
(1) Prepare ordinary nutrient agar (1.0 to 1.5%) with a reaction of +1.0.
(2) Tube in 1.0 to 3.0 cc. lots.
(3) Tube well separated milk in 1.0 to 3.0 cc. quantities.
(4) Mix one part serum (sheep, beef, horse or rabbit) with three parts distilled water.
(5) Tube (4) in 1.0 to 3.0 cc. quantities.
(6) When ready for use melt the sterile agar tubes and cool to 45° to 50°C. in a water bath.
(7) Heat sterile tubes of (3) and (4) in the water bath and add one tube of (3) and one tube of (4) to a tube of agar (equal parts agar, milk and serum).
(8) Mix thoroughly.

Sterilization: Sterilize (2), (3) and (5) in the autoclave.
Use: Isolation of *B. diphtheriae*. The authors used this medium in preparing "little plate" cultures on slides.
Reference: Frost, Charlton and Little (1921 p. 30).

2015. Salomon's Basal Ascitic Fluid Agar

Constituents:

1. Nutrient agar (3.0%)	100.0 cc.
2. Ascitic fluid	50.0 cc.
3. Litmus tincture	

Preparation:
(1) Prepare 3.0% nutrient agar.
(2) Distribute (1) into 10.0 cc. lots.
(3) Prepare a 10.0% solution of one of the added nutrients in litmus tincture.
(4) Add 1.5 cc. of one of (3) to 10.0 cc. of melted (2) cooled to 58°C.
(5) Add 5.0 cc. of ascitic fluid, heated to 58°C. to each tube of (4).

Sterilization: Not specified.
Use: To study fermentation of streptococci. Author gave the following reactions:
A. *Strept. pyogenes* group.
  I. *Strept. pyogenes*: Acid formation from soluble amylum. Mannitol and raffinose unchanged.
  II. Strains from blood. Acid formation from glycerol and mannitol.
B. *Strept. mucosus.*
  I. Acid from glycerol, arabinose and mannitol. Raffinose and soluble amylum unchanged.
  II. Do not attack any of the sugars in 24 hours and rarely after 48 hours.
C. Pneumococci formed no acid on carbohydrate litmus ascitic agar.

Similar media were used by other investigators to study fermentation of other cocci.

Added nutrients: The author used one of the following materials:

glycerol
erythritol
arabinose
adonitol
isodulcitol
mannose
glucose
mannitol
galactose
levulose
dulcitol
lactose
raffinose
inulin
maltose
sucrose
dextrin
amylum (soluble)

Variants:
(a) Symmers and Wilson cultivated meningococci on a similar medium prepared as follows:
  (1) Prepare nutrient agar containing 3.0% Chapoteaut's peptone.

(2) Add 1.0% sugar and some litmus solution to (1).
(3) Steam for 10 minutes on 3 successive days to sterilize.
(4) Add one part of sterile ascitic fluid to two parts (3).
(5) Tube and slant.
(6) Incubate 2 days to test sterility.

(b) Gordon (Abel) used a medium prepared as follows to study fermentation of meningococci and gonococci.
(1) Prepare 10.0% solution of any desired carbohydrate, alcohol, etc., in Kahlbaum's litmus solution and boil two minutes.
(2) When cool add 0.5 cc. of normal soda solution to each 10.0 cc. of (1).
(3) Mix 3 parts 3.0% nutrient agar with one part ascitic fluid.
(4) Add 1.5 cc. of (2) to each 13.5 cc. of (3).
(5) Pour into plates.

(c) v. Przewoski used the following medium to determine fermentation ability of diphtheria and pseudo diphtheria:
(1) Prepare nutrient agar.
(2) To 100.0 cc. of liquid (1) add 5.0 cc. litmus solution (Kahlbaum), 2.0 cc. ascitic fluid and 1.0 g. of one of the following:

fructose
glucose
mannose
dulcitol
mannitol
lactose
inulin

(d) Hancken studied the fermentation ability of meningococci on a medium prepared as follows:
(1) Prepare nutrient agar.
(2) Dissolve 2.5 g. of any desired carbohydrate sugar or alcohol in 50.0 cc. of Kahlbaum's litmus solution. Boil for a few minutes.
(3) Melt the sterile agar and cool to 55°C. and add 3.3 cc. ascitic fluid and 4.0 cc. of (2) to 9 or 10.0 cc. of the agar under aseptic conditions.
(4) Pour into sterile plates.

(e) Eastwood studied the fermentation of carbohydrates, alcohols, etc., on Lingelsheim's medium prepared as follows:
(1) Prepare a 10.0% solution of one of the following: maltose, glucose, levulose, galactose, mannitol, dulcitol, sucrose, lactose, inulin with Kubel-Tiemann's litmus and sterilize. (Method not given.)
(2) Add (1) to ascitic agar so that the agar content is 1.0%.
(3) Final sterilization not given.

Eastwood reported that meningococci gave acid (red colonies) with glucose and maltose only.

References: Salomon (1908 p. 2), Symmers and Wilson (1909 p. 10), Abel (1912 p. 159), v. Przewoski (1912 p. 15), Hancken (1916 p. 368), Eastwood (1916 p. 408).

2016. Rosenow's Glucose Ascitic Fluid Agar

Constituents:

1. Nutrient agar (2.0%).	1000.0 cc.
2. Glucose..............	2.0 to 10.0 g.
3. Ascitic fluid.........	300.0 cc.

Preparation:
(1) Prepare 2.0% nutrient agar with a reaction of 0.4 to 0.6% acid to phenolphthalein.
(2) Dissolve 2 in (1).
(3) Tube in 7.0 to 8.0 cc. lots.
(4) Boil sterile (3) to drive off oxygen and cool to 50°C.
(5) Add 2 to 3.0 cc. heated ascitic fluid (60°C. for 24 hours) to each tube.
(6) Cool to 40°C.

Sterilization: Method of sterilization of (3) not given. See step (5) above for sterilization of ascitic fluid.

Use: To isolate organism causing rheumatism, author added some of rheumatic material to medium and mixed well; plunged tube into cold water to "set" and incubated at 37°C. Author reported that largest number of colonies developed between 1.5 cm. from the top and 3.5 cm. from the bottom. A similar medium was used for the cultivation of meningococci and gonococci by Tilmant and Carrien.

Variant: Tilmant and Carrien cultivated meningococci and gonococci on a medium prepared as follows:
(1) Collect about 100.0 cc. of ascitic fluid as carefully as possible.
(2) Estimate the albumin content and dilute with physiological saline so that there will be 3.0 g. of albumin per liter.
(3) Add 1.0 cc. of a 10.0% soda solution

for each 200.0 cc. of fluid and sterilize in the autoclave.

(4) Add sterile (3) to sterile melted glucose agar (or gelatin) in proportion of 3 to 7.

References: Rosenow (1914 p. 61), Tilmant and Carrien (1917 p. 499) taken from (1917 p. 222).

2017. Ruediger's Inulin Ascitic Fluid Agar

Constituents:

1. Distilled water	200.0 cc.
2. Peptone (Witte)	10.0 g.
3. Agar	15.0 g.
4. Bouillon (sugar free)	800.0 cc.
5. Inulin	15.0 g.
6. Litmus (5.0% soln.)	20.0 cc.
7. Ascitic fluid	145.0 cc.

Preparation:

(1) Dissolve 2 and 3 in sugar free bouillon by boiling for one hour. Add water to make the volume to 800.0 cc.

(2) Heat in autoclave for 15 to 20 minutes.

(3) Clarify with egg.

(4) Filter thru cotton.

(5) Make up volume to 800.0 cc. with distilled water.

(6) Dissolve 15.0 g. pure inulin in 200.0 cc. distilled water.

(7) Mix (6) with (5).

(8) Add 20.0 cc. of 5.0% solution of litmus (Merck's highest purity).

(9) Tube in 7.0 to 8.0 cc. lots.

(10) Just before use, melt sterile agar, cool to 45° and add 1.0 cc. of heated (65°) fluid to each tube.

Sterilization: Sterilize (9) in autoclave under 10 pounds pressure for 15 minutes.

Use: Isolation of pneumococci. Acid production. Ruediger reported the pneumococci colonies were red.

Reference: Ruediger (1906 p. 184).

2018. Klimenko's Glycerol Ascitic Fluid Agar

Constituents:

1. Nutrient agar (3.0 or 4.0%)	100.0 cc.
2. Glycerol (1.0%)	10.0 cc.
3. Ascitic fluid (human)	100.0 cc.

Preparation:

(1) Prepare 3.0 or 4.0% nutrient agar containing 1.0% glycerol.

(2) Mix equal parts of (1) and human ascitic fluid (or blood serum).

Sterilization: Not specified.

Use: Cultivation of whooping cough bacilli. Author reported that whooping cough bacilli grew as a glistening opalescent covering of a grayish shade.

Variants:

(a) Cantani prepared a similar medium as follows:

(1) Mix equal amounts of glycerol and the albuminous fluid (urine, pus, milk, egg white, etc.). Place in an Erlenmeyer flask.

(2) Storing the fluid in glycerol tends to sterilize the fluid.

(3) After a time test the sterility of the mixture.

(4) Prepare nutrient agar.

(5) Mix 6 parts ascitic fluid (with or without glycerol) with one part of (3).

(6) Add to each tube of sterile (4) 0.5 to 0.75 cc. of (5).

(7) Incubate 24 hours to test sterility.

(b) Cantani (Besson) prepared a medium as follows:

(1) Mix equal parts of sterile ascitic fluid and sterile glycerol.

(2) Allow to stand several hours.

(3) Add 0.5 to 0.75 cc. of (2) to tubes of liquified sterile agar.

References: Klimenko (1909 p. 311), Cantani (1910 p. 472), Besson (1920 p. 54).

2019. Vellion's Ascitic Fluid Agar

Constituents:

1. Nutrient agar (2.0%).
2. Ascitic fluid.

Preparation:

(1) Prepare 2.0% nutrient agar with a slightly alkaline reaction.

(2) Tube.

(3) Cool melted sterile (2) to about 60°C.

(4) Add an equal amount or even $\frac{1}{3}$ amount of ascitic fluid.

(5) Slant.

Sterilization: Method of sterilization of (2) not given.

Use: Cultivation of gonococci.

Variants:

(a) Thoinot and Masselin prepared the medium as follows:

(1) Liquify sterile agar and cool to about 50°C.

(2) Add a known quantity of sterile

ascitic fluid to (1) under aseptic conditions.

(b) Besson prepared the medium as follows:
  (1) Melt tubes of sterile nutrient agar.
  (2) Cool to 45 to 50°C.
  (3) Add one-half or one-third the volume of sterile ascitic fluid to each tube.
  (4) Mix thoroly by rolling the tube between the palms of the hands.
  (5) Slant or pour into a Petri dish.

(c) Dopter and Sacquépée prepared the medium as indicated:
  (1) Add 0.5 to 1.0 cc. of sterile ascitic fluid to agar slants.
  (2) Incline so that the entire surface is covered with the serum.
  (3) Allow to stand for 12 to 24 hours.

References: Vellion (1898 p. 24), Thoinot and Masselin (1902 p. 36), Besson (1920 p. 53), Bezançon (1920 p. 119), Dopter and Sacquépée (1921 p. 138), Klimmer (1923 p. 226), Stitt (1923 p. 42).

2020. Herrold's Phosphate Ascitic Fluid Agar

Constituents:

1. Nutrient agar	300.0 cc.
2. Na_2HPO_4	
3. Ascitic fluid	100.0 cc.

Preparation:
(1) Prepare nutrient agar substituting Na_2HPO_4 for NaCl.
(2) Heat ascitic fluid at 56°C. for one hour.
(3) Add one part heated ascitic fluid to three parts melted (1).
(4) Tube or pour into plates.

Sterilization: Not specified.

Use: Cultivation of streptococci for agglutination and absorption studies.

Reference: Herrold (1922 p. 80).

2021. Loeffler's Dye Bile Agar (Abel)

Constituents:

1. Nutrient agar (3.0%)	1000.0 cc.
2. Nutrose	10.0 g.
3. Bile	30.0 cc.
4. Safranin (0.2% soln.)	10.0 cc.
5. Pure blue (1.0% soln.)	30.0 cc.
6. Malachite green (0.2% soln.)	30.0 to 40.0 cc.

Preparation:
(1) Add 5.0 cc. of normal NaOH to a liter of neutral 3.0% agar.
(2) Add 100.0 cc. of a 10.0% nutrose solution to sterile (1).
(3) When ready for use liquify the agar and cool to 45°C.
(4) To each 100.0 cc. of (4) add 3.0 cc. of sterile filtered ox bile, 1.0 cc. of a 0.2% aqueous "Pure Safranin" (Grübler), 3.0 cc. of a 1.0% aqueous "Pure blue double concentrated" (Höchster Farbwerke), and 3.0 to 4.0 cc. of a 2.0% aqueous malachite green crystals (Höchst.). Mix thoroly.
(5) Pour in plates.

Sterilization: Method of sterilization of agar not given. Sterilize the bile by boiling.

Use: Enrichment of typhoid group. Klimmer reported that colon colonies were inhibited. Paratyphoid colonies grew luxuriantly as glassy, milk-like colonies.

References: Abel (1912 p. 132), Klimmer (1923 p. 214).

2022. Hasting's Milk Agar

Constituents:

1. Nutrient agar	1000.0 cc.
2. Milk	100.0 to 120.0 cc.

Preparation:
(1) Prepare nutrient agar.
(2) Melt (1) and cool to 50°C.
(3) Add 10.0 to 12.0% skimmed milk to (2).
(4) Tube and slant or pour in plates.

Sterilization: Not specified.

Use: To demonstrate proteolysis. Hastings reported that if proteolysis takes place the opaque medium clears. More advantageous than gelatin to determine proteolysis for milk agar may be incubated at a temperature greater than 20°C. Used also to cultivate fowl diphtheria bacilli and *B. vulgaricus*.

Variants:

(a) Müller reported the colonies of fowl diphtheria bacilli appeared after 40 hours when inoculated on the following medium:
  (1) Prepare nutrient agar.
  (2) Melt (1) and mix equal parts of melted agar and skim milk at 100°C.
  (3) Pour into sterile tubes or plates.

(b) Stitt cultivated *B. bulgaricus* on a medium prepared by adding 2.0 or 3.0 cc. of plain or litmus milk to a tube of melted nutrient agar.

References: Hastings (1903 p. 384), Müller (1906 p. 521), Tanner (1919), Klimmer (1923 p. 203), Stitt (1923 p. 42).

2023. Valleti's Whey Agar

Constituents:

1. Nutrient agar.
2. Whey.

Preparation:

(1) Prepare nutrient agar.

(2) Prepare serum from cow's milk by boiling milk with a few drops of acetic acid.

(3) Add 2.0 cc. of milk serum to agar (amount not given).

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli (especially bovine types). Author reported that the bovine type developed after about 36 hours.

References: Valleti (1913 p. 240), Wigger (1914 p. 3).

2024. Klimmer's Casein Agar

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	0.1 g.
4. $MgSO_4$	0.3 g.
5. K_2HPO_4	0.3 g.
6. Casein	5.0 g.
7. Agar (2.0%)	1000.0 cc.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Mix equal parts sterile (1) and liquid sterile 2.0% agar.

(3) Pour into plates.

Sterilization: Not specified.

Use: Determine peptonizing ability. The author reported that peptonizing bacteria dissolved the casein giving a light area.

Reference: Klimmer (1923 p. 203).

2025. Mayer's Mucin Agar

Same as medium 969, but substituting nutrient agar for bouillon.

2026. Fichtner's Sputum Agar

Constituents:

1. Nutrient agar.
2. Sputum.

Preparation:

(1) Obtain clean sputa and place in sterile test tubes.

(2) Immerse the tubes in a water bath so that the water level in the bath is higher than the sputa in the tube. Do not heat over 70°C. Usually 60°C. is sufficient.

(3) Heat the sputa until a homogenous thin liquid is formed. Shake or stir the sputa occasionally. Do not heat longer than 1½ to 2 hours.

(4) Prepare slants or plates from nutrient agar.

(5) Streak the surface of agar slants or plates with the sterilized sputa, in the same manner as one streaks agar with blood to prepare blood agar slants.

Sterilization: See preparation of sterilization of sputum. Method of sterilization of agar not given.

Use: Cultivation of influenza bacilli. Author reported that influenza colonies were generally more turbid and flatter than on blood agar.

Reference: Fichtner (1904 p. 376).

2027. Finger, Ghon and Schlagenhaufer's Urine Agar

Constituents:

1. Nutrient agar (2.0%).
2. Urine.

Preparation:

(1) Prepare 2.0% nutrient agar containing 1.0% peptone. Reaction to be neutral or slightly alkaline.

(2) Obtain urine under aseptic conditions. The reaction is slightly acid.

(3) Add one part sterile (2) to two parts (1). The final reaction is neutral.

Sterilization: Method of sterilization of agar not given.

Use: Cultivation of gonococci.

Variants:

(a) The authors reported that the urine may be mixed with the agar and then sterilized. (Method not given.) The urine may be sterilized by heating at 70 to 80°C. for 30 minutes and then mixing one part urine with two parts sterile agar. The finished medium reacts neutral or slightly alkaline.

(b) Hammer specified the use of albumin urine from a nephritis patient.

References: Finger, Ghon and Schlagenhaufer (1894 p. 14), Hammer (1895 p. 859).

2028. Eberson's Yeast Infusion Agar

Constituents:

1. Water	100.0 cc.
2. Nutrient agar (1.0%, with 2.0% peptone)	100.0 cc.
3. Potassium phosphate (0.4%)	0.4 g.
4. Yeast, bakers or brewers	10.0 g.

Preparation:

(1) Macerate 10.0 g. of bakers or brewers yeast in 100.0 cc. of water for 20 minutes.

(2) Steam the suspension for 2 hours at a temperature not exceeding 100°C.

(3) Clarify by adding Merck's dialized iron (5.0% ferric hydroxide) and filtering thru glass wool.

(4) Add an equal amount of 1.0% agar containing 2.0% peptone and 0.4% potassium phosphate to (3).

(5) Adjust the reaction to pH 7.6 (pH 7.4 following sterilization).

(6) Tube in 10.0 cc. quantities.

Sterilization: Autoclave for 30 minutes at 15 pounds pressure.

Use: Culture medium for preserving stock cultures of meningococci.

Reference: Eberson (1919 p. 852).

SUBGROUP II-C. SECTION 7

Basal or complete media containing agar and a digest or autolysate other than a commercial digest.

A_1. Digested by acids or bases.
  Robinson and Rettger's Lactalbumin Agar 2029
  Robinson and Rettger's Casein Edestin Derivatives. Lactalbumin Agar 2030
  Robinson and Rettger's Casein Product C Agar 2031
  Robinson and Rettger's Casein Product B Agar 2032
  Robinson and Rettger's Edestin Product Agar 2033
  Deycke and Vöigtlander's Albuminate Agar 2034
A_2. Digested by enzymes.
B_1.* Enzymes of animal origin employed.
C_1. Containing peptic digests only.
D_1. Digested by means of minced stomach.
E_1. Not containing animal fluids.
  Martin and Loiseau's Peptic Digest Agar 2035
  Dujarric's Orange Juice Peptic Digest Agar 2036
  Stickel and Meyer's Peptic Digest Agar 2037
  Harvey's Basal Peptic Digest Agar 2037a
  Besredka and Jupille's Egg Stomach Digest Agar 2038
E_2. Containing animal fluids.
  Sellards and Bigelow's Veal Digest Blood Agar 2039
  Martin's Basal Stomach Digest Veal Infusion Agar 2040
  Harvey's Defibrinated Blood Digest Agar 2041
  Harvey's Pepsin Digest Serum Agar. 2042
  Martin's Digest of Ascitic Fluid Agar 2043
D_2. Digested by means of pepsin.
  Harvey's Pepsinized Blood Agar... 2044
  Deycke and Vöigtlander's Pepsinized Meat Agar 2045
  Kammen's Pepsinized Yeast Agar (Klimmer) 2046
  Jensen's Milk Digest Agar 2047
  Bacto Nutritive Caseinate Agar (Dehydrated) 2048
  Bacto Peptonized Milk Agar (Dehydrated) 2049
C_2.* Containing tryptic digests only.
D_1.† Containing digests of casein or milk products.
E_1. Additional constituents, if any, inorganic.
  Cole and Onslow's Trypsinized Casein Agar 2050
  Teruuchi and Hida's Trypsinized Casein Agar 2051
  Norris' Trypsinized Caseinogen Agar 2052
  Norris' Simplified Caseinogen Agar. 2053
  Teague and Deibert's Trypsinized Casein Agar 2054
E_2. Containing additional organic constituents.
  Sierakowski's Trypsinized Casein Lactose Agar 2055
  Harvey's Trypsinized Caseinogen Blood Agar 2056
  Harvey's Blood Tryptamine Agar.. 2057

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* See page 651 for B_2.

* See C_3, page 651.

† See page 651 for D_2.

- Teague and Deibert's Serum Trypsinized Casein Agar.............. 2058
- Kulp and Rettger's Trypsinized Casein Agar..................... 2059
- Kulp and Rettger's Trypsinized Klim Agar...................... 2060

D_2. Not containing digests of casein or milk products.

E_1. Containing digests of mutton or other meats.

- Harvey's Trypsinized Ox Heart Agar............................ 2061
- Norris' Trypsinized Mutton Agar.. 2062
- Harvey and Iyengar's Desiccated Trypsinized Mutton Bouillon Agar........................... 2063
- Harvey and Iyengar's Desiccated Bile Salt Trypsinized Mutton Agar............................ 2064
- Vardon's Desiccated Aronson's Agar............................ 2065
- Deycke and Voigtländer's Tryptic Meat Agar....................... 2066
- Murray and Ayrton's Heart Infusion Agar....................... 2067
- Hottinger's Agar (Park, Williams and Krumwiede)................. 2068
- Gordon and Hines' Trypagar....... 2069

E_2. Containing other than meat digests.

- Duval and Harris' Tryptic Digest Agar........................... 2070
- Distaso's Trypsinized Serum Agar. 2071
- Stickel and Meyers' Trypsinized Blood Clot Agar................. 2072

C_3. Containing both tryptic and peptic digests.

- Stickel and Meyers' Tryptic and Peptic Digest Agar.............. 2073
- Deycke and Voigtländer's Tryptic and Peptic Meat Digest Agar.... 2074

B_2. Enzymes of plant origin employed.

- Kligler's Yeast Autolysate Agar... 2075
- van Steenberge's Yeast Extract Agar........................... 2076
- Jötton's Yeast Autolysate Agar.... 2077
- Couret and Walker's Autolyzed Tissue Agar....................... 2078

2029. Robinson and Rettger's Lactalbumin Agar

Constituents:

1. Water	1000.0 cc.
2. Lactalbumin	20.0 g.
3. Agar	15.0 g.

Preparation:

(1) Heat 100.0 g. of crude commercial milk albumin containing equal amounts of calcium phosphate and protein for 2 to 3 hours with 200.0 cc. of 10.0% HCl under reflex condenser.
(2) Filter and heat residue with 200.0 cc. 10.0% HCl until no Biuret is given.
(3) Heat in open vessel to drive off HCl.
(4) Neutralize with NaOH.
(5) Filter.
(6) Evaporate filtrate to sticky paste. This is lactalbumin. Add paste and agar to water. Adjust to faintly alkaline with litmus. Sterilize. (Method not given.)
(7) Dissolve 20.0 g. of (6) and 15.0 g. agar in 1000.0 cc. of water.
(8) Make faintly alkaline to litmus.

Sterilization: Method not given.

Use: General culture media for pathogenic forms. Authors reported that 2.0% lactalbumin gave best results; a 4.0% medium contained too much NaCl, due to neutralization of acid by NaOH.

Reference: Robinson and Rettger (1918 p. 220).

2030. Robinson and Rettger's Casein Edestin Derivatives, Lactalbumin Agar

Constituents:

1. Water	1000.0 cc.
2. Casein product C	25.0 g.
3. Edestin product	2.5 g.
4. Lactalbumin product (paste)	2.5 g.
5. Agar	15.0 g.

Preparation:

(1) Prepare casein product C as follows:
  (a) Boil over free flame under a reflux condenser for 8 hours, 50.0 g. crude casein in 200.0 cc. 10.0% HCl.
  (b) Distill solution by frequently adding water.
  (c) Neutralize residue with NaOH.
(2) Prepare edestin product by:
  (a) Heat 40.0 g. pure re-crystallized edestin over a free flame with 10.0% HCl under a reflux condenser, until it no longer gives Biuret test.
  (b) Heat for several hours in open dish to drive out HCl.
  (c) Neutralize with NaOH.

(3) Prepare lactalbumin product as follows:
  (a) Heat for 2 to 3 hours 100.0 g. crude commercial milk albumin containing equal amounts of calcium phosphate and protein, with 200.0 cc. of 10.0% HCl.
  (b) Filter off residue of protein and heat with 200.0 cc. of 10.0% HCl until no Biuret is obtained.
  (c) Heat in open vessel to drive off HCl.
  (d) Neutralize (3) with NaOH and filter.
  (e) Use part of filtrate as such and evaporate rest to paste.
(4) Dissolve 25.0 g. casein product C, 2.5 g. Edestin product, and 2.5 g. lactalbumin product paste, and 15.0 g. agar in water.
(5) Make faintly alkaline to litmus.

Sterilization: Method not given.

Use: General culture medium for pathogenic forms.

Reference: Robinson and Rettger (1918 p. 220).

2031. Robinson and Rettger's Casein Product C Agar

Constituents:
1. Water...................... 1000.0 cc.
2. Casein product C.......... 30.0 g.
3. Agar...................... 15.0 g.

Preparation:
(1) Boil 50.0 g. of crude casein over a free flame in 200.0 cc. of a 10.0% HCl solution under a reflux condenser for 8 hours.
(2) Distill the solution by adding water frequently.
(3) Neutralize the residue by adding NaOH.
(4) Add 30.0 g. of (3) and 15.0 g. of agar to 1000.0 cc. of water.
(5) Make slightly alkaline to litmus.

Sterilization: Method not given.

Use: General culture medium for pathogenic forms.

Reference: Robinson and Rettger (1918 p. 220).

2032. Robinson and Rettger's Casein Product B Agar

Constituents:
1. Water...................... 1000.0 cc.
2. Casein product B.......... 15.0 g.
3. Agar...................... 15.0 g.

Preparation:
(1) Boil over a free flame 40.0 g. of crude casein with 200.0 cc. of 9.0% HCl under a reflux condenser.
(2) Heat in an open dish until no more acid vapor comes off.
(3) Evaporate to a thick paste. This is casein product B.
(4) Dissolve 15.0 g. of (3) and 15.0 g. of agar in 1000.0 cc. of water.
(5) Make faintly alkaline to litmus by the addition of litmus.

Sterilization: Not specified.

Use: General culture medium for pathogenic forms.

Variants:
(a) The casein product may be decolorized with animal charcoal if desired.
(b) Author added 5.0 g. of Liebig's meat extract with 10.0 g. starch to the medium.

Reference: Robinson and Rettger (1918 p. 220).

2033. Robinson and Rettger's Edestin Product Agar

Constituents:
1. Water...................... 1000.0 cc.
2. Edestin product........... 50.0 g.
3. Agar...................... 15.0 g.

Preparation:
(1) Prepare edestin product as follows:
  (a) Heat over a free flame under a reflux condenser 40.0 g. pure recrystallized edestin and 10.0% HCl until a Biuret test is no longer given.
  (b) Heat in an open dish to remove as much HCl as possible.
  (c) Decolorize with animal charcoal.
(2) Dissolve 50.0 g. of (1) and 15.0 g. of agar in 1000.0 cc. of water.
(3) Neutralize by the addition of NaOH.

Sterilization: Method not given.

Use: General culture medium for pathogenic forms. The addition of meat extract improves the medium.

Variants: The authors added 5.0 g. Liebig's meat extract to the medium.

Reference: Robinson and Rettger (1918 p. 220).

2034. Deycke and Voigtländer's Albuminate Agar

Constituents:

1. Water	3000.0 cc.
2. Meat, horse	200.0 g.
3. NaOH (3.0%)	250.0 cc.
4. NaCl	7.5 g.
5. Glycerol	150.0 g.
6. Agar	

Preparation:

(1) Place 200.0 g. of finely chopped fat free horse meat in 3.0% NaOH solution (made by adding 30.0 g. of fused, pure caustic soda to 1 liter of water) and grind fine.
(2) Place in an Erlenmeyer flask and incubate at 37°C. for 24 to 30 hours until solution is complete.
(3) Add HCl to the filtrate until neutral to litmus.
(4) Dilute to 3 liters with water.
(5) Add 7.5 g. NaCl, 150.0 g. glycerol and make alkaline wth soda solution.
(6) Dissolve agar in (5) to solidify.

Sterilization: Not specified.

Use: Cultivation of parasitic and saprophytic forms. Add glucose for the cultivation of streptococci. Reitz used this medium in the bacteriological examination of butter for diphtheria bacilli.

Variants: The authors added 1.5 to 2.0% glucose.

References: Deycke and Voigtländer (1901 p. 624), Reitz (1906 p. 723).

2035. Martin and Loiseau's Peptic Digest Agar

Constituents:

1. Water	500.0 cc.
2. Beef	250.0 g.
3. Martin's Stomach Digest Solution	
4. Agar	8.0 g.
5. Glucose	15.0 g.
6. KNO_3	2.0 g.

Preparation:

(1) Prepare an extract of 250.0 g. of beef in 500.0 cc. water. Method not given.
(2) Mix 500.0 cc. of (1) with 500.0 cc. of Martin's bouillon (see medium 998).
(3) To a liter of the mixture add 4, 5 and 6.
(4) After solution is complete, add the white of an egg and heat at 115° for 30 minutes.
(5) Filter.
(6) Distribute in sterile tubes to a depth of 10 to 12 cm.

Sterilization: Sterilize at 100° for 30 minutes on 3 days or 115° for 30 minutes.

Use: Isolation of diphtheria bacillus.

Variants: Harvey prepared a similar medium as follows:

(1) Mince finely fat-free veal.
(2) Add 500.0 g. of (1) to 1000.0 cc. distilled water or clear tap water, and heat for 20 minutes over a free flame at a temperature not to exceed 50°C., or simply keep in a cool place over night.
(3) Skim off the fat floating on the surface.
(4) Boil 10 minutes.
(5) Filter thru wet, thick clean cloth.
(6) Add 5.0 g. NaCl to the filtrate.
(7) Steam 45 minutes.
(8) Make up the volume to 1000.0 cc.
(9) Adjust the reaction.
(10) Steam 30 minutes.
(11) Filter while hot thru well wetted thick filter paper.
(12) Distribute into flasks.
(13) Mix 1000.0 cc. of (12) and 1000.0 cc. of Martin's Stomach Digest Solution (see medium 998, variant (c)).
(14) Dissolve 30.0 g. glucose, 4.0 g. KNO_3 and 6.0 g. agar in (13).
(15) Sterilization not specified.

References: Martin and Loiseau (1916 p. 678), Harvey (1921–22 p. 100).

2036. Dujarric's Orange Juice Peptic Digest Agar

Constituents:

1. Martin's Stomach Digest Solution	75.0 cc.
2. Orange juice	125.0 cc.
3. Agar	

Preparation:

(1) Filter orange juice thru paper.
(2) Mix 125.0 cc. of (1) with 75.0 cc. Martin's bouillon. (See medium 998).
(3) Add agar to solidify as in the usual way. (Method not given.)
(4) The reaction is acid. The reaction may be adjusted to any desired alkalinity by the addition of soda.

Sterilization: Not specified.

Use: General culture medium.

Reference: Dujarric (1916 p. 843).

2037. Stickel and Meyer's Peptic Digest Agar

Constituents:

1. Water, tap	4000.0 cc.
2. Pig's stomach, minced	400.0 g.
3. Liver, minced (beef placenta or blood clots)	400.0 g.
4. HCl (Baker Chemical Co.).	40.0 g.
5. K_2HPO_4	8.0 g.
6. Agar	80.0 g.

Preparation:

(1) Wash clean and mince fine five or more large pigs' stomachs. Mix an equal amount of clean pig or beef liver, cheap fat-free beef, placenta or blood clots.

(2) Mix 2, one of 3, and 4 in 1 at 50°C. and keep at 50°C. for 18–24 hours.

(3) Make a Biuret and tryptophane test. When both are + the digest is yellowish green and contains very little undigested debris.

(4) Transfer to large bottles and steam for 10 minutes at 100°C. to stop digestion.

(5) Strain thru cotton or preferably store over night in the ice chest and decant after 24 hours.

(6) Warm (5) to 70°C. and neutralize with 2N Na_2CO_3 to litmus.

(7) Add 5 and 6 to (6).

(8) Autoclave at 10 pounds pressure for 45 minutes or heat in double boiler to 100°C. until the agar is dissolved.

(9) Restore the volume lost by evaporation.

(10) Adjust to slightly alkaline to litmus or pH = 7.3 by using 2N KOH or NaOH.

(11) Cool to 60°C. and add white of egg with crushed shells or for sake of economy, ordinary beef or sheep serum in the quantity of 25–50 cc. per liter.

(12) Autoclave for one hour at 115°C.

(13) Filter thru cotton and distribute in 200–500 cc. quantities.

Sterilization: Sterilize at 100° for 30 minutes on 2 successive days or at 10 pounds pressure for 15 minutes.

Use: General inexpensive culture medium.

Variants:

(a) The authors prepared a sugar free medium as follows:

Carry out step (1) thru (6) as given above.

(7) Sterilize at 10 pounds pressure for 15 minutes in the autoclave or for 30 minutes at 100°C. on 2 successive days.

(8) Inoculate (7) with 1.0% of a 24 hour old broth culture of *B. saccharolyte* or *B. coli* and incubate for 12–18 hours at 37°C.

(9) Steam 20 minutes.

(10) Adjust to desired reaction.

(11) Add 5 and 6 to (10).

(12) Autoclave at 10 pounds pressure for 45 minutes or heat in double boiler to 100°C. until the agar is dissolved.

(13) Restore the volume lost by evaporation.

(14) Adjust to slightly alkaline to litmus or pH = 7.3 by using 2N KOH or NaOH.

(15) Cool to 60°C. and add white of egg with crushed shells or for sake of economy, ordinary beef or sheep serum in the quantity of 25–50 cc. per liter.

(16) Autoclave for one hour at 115°C.

(17) Filter thru cotton and distribute in 200.0 to 500.0 cc. quantities.

(18) Sterilize at 100° for 30 minutes on 2 successive days or at 10 pounds pressure for 15 minutes.

(b) Stickel and Meyer prepared a medium as follows:

(1) Obtain 10 liters fresh beef blood from the abattoir.

(2) Decant and store the serum (which has separated on standing) in a refrigerator.

(3) Weigh the blood clots and mix 100.0 g. with 1 liter tap water.

(4) Place mixture in an enameled pot, bring slowly to a boil and boil slowly for 5 minutes, stirring constantly.

(5) Wash and mince pig's stomach.

(6) Cool (4) to 50°C. and add 100.0 g. minced pig's stomach for each liter of (4).

(7) Transfer to glass or porcelain receptacle and finally add 1.0% HCl.

(8) Digest at 50°C. for 18–24 hours.

(9) Make a biuret and tryptophane test, when both are +, the digest is yellowish green and contains very little undigested debris.
(10) Transfer to large bottles and steam for 10 minutes to stop digestion.
(11) Strain thru cotton or preferably store over night in ice chest and decant after 24 hours.
(12) Warm (11) to 70°C. and neutralize with 2N Na_2CO_3 to litmus.
(13) Filter into a flask.
(14) Add 5 and 6 to (13).
(15) Adjust to desired reaction using litmus or preferably to a definite H-ion concentration (pH = 7.0 to 7.5).
(16) Clear (15) by adding 5–10.0% of the decanted beef serum. Steam 45 to 60 minutes.
(17) Remove (16) from steamer and allow the clot to form as a compact mass. Decant or better centrifuge the medium to remove it.
(18) Sterilize at 100° for 30 minutes on 2 successive days.

Reference: Stickel and Meyer (1918 pp. 79, 81).

2037a. Harvey's Basal Peptic Digest Agar

Constituents:

1. Distilled water.
2. Veal, fat free.
3. Minced stomach.
4. HCl.
5. Agar.

Preparation:

(1) Clean and wash a number of pigs' stomachs.
(2) Mince (1) finely.
(3) Add 10.0 cc. of strong hydrochloric acid to 1000 cc. water at 50°C.
(4) Add 200 g. (2) to (3) and keep at 50°C. for 20 hours.
(5) Raise the temperature to the boiling point.
(6) Pour the mixture on a thick clean cloth. Collect the fluid and that obtained by squeezing the cloth and its contents.
(7) Heat the fluid to 80°C. and make faintly alkaline to litmus while at 80°C.
(8) Filter thru well-wetted thick filter paper while hot.
(9) Steam 30 minutes.
(10) Filter again thru well-wetted thick filter paper.
(11) Mince finely, fat free veal, and add 500 g. to 1000 cc. water.
(12) Incubate for 18 hours at 37°C.
(13) Pour the mixture on a thick clean cloth.
(14) Collect the fluid, and that obtained by squeezing the cloth and its contents.
(15) Mix (14) with an equal volume of pepsin digest solution (10).
(16) Heat to 70°C.
(17) Make the reaction neutral to litmus.
(18) Add 7 cc. of normal NaOH per liter.
(19) Filter thru well-wetted thick filter paper.
(20) Add sufficient agar to give a solid medium.
(21) Steam 30 minutes.
(22) Filter while hot thru well-wetted thick filter paper.

Sterilization: Method of sterilization of the agar base not given.

Added nutrients: The author used the agar base in the following manner:

(A) Defibrinated blood.
  (1) Mix one part defibrinated blood with three parts of distilled water.
  (2) Add 0.5 cc. of 10% NaOH to 100 cc. of (1).
  (3) Sterilize (2) in the autoclave at 112°C.
  (4) Mix one part (3) with two parts sterile agar, base at 80°C. under aseptic conditions.
  (5) Slant.
(B) Formalinized Serum.
  (1) Add 1 cc. formalin to 500 cc. horse serum.
  (2) Add 1% ammonia solution to (1) to neutralize to litmus.
  (3) Mix one part (2) with two parts distilled water.
  (4) Sterilize at 110°C. for 15 minutes.
  (5) Mix one part (4) with three parts sterile agar base.

Use: Isolation and cultivation meningococcus.

Reference: Harvey (1921–22, p. 74, 81).

2038. Besredka and Jupille's Egg Stomach Digest Agar

Constituents:

1. Distilled water
2. Beef or veal.................. 750.0 g.
3. Egg
4. Stomach (hog)
5. Agar........................ 2%

Preparation:

(1) Macerate 750.0 g. beef in 1200.0 cc. water.
(2) Heat slowly at first and then boil for 30 minutes.
(3) Concentrate to one liter.
(4) Filter and dissolve 2.0 g. of agar for every 100.0 cc. of filtrate.
(5) Make slightly alkaline and boil for 25 minutes.
(6) Filter and distribute in tubes.
(7) Add 4.0 cc. of egg stomach digest solution (see medium 1007 for preparation) to each tube of sterile (6). (Ordinary peptone agar may be used.)

Sterilization: Sterilize (6) at 115°.

Use: Cultivation of gonococcus, whooping cough bacillus, pneumococci, tubercle bacilli and other forms difficult to cultivate.

Reference: Besredka and Jupille (1914 p. 577).

2039. Sellards and Bigelow's Veal Digest Blood Agar

Same as medium 1000 but solidified by the addition of 2.0% agar.

2040. Martin's Basal Stomach Digest Veal Infusion Agar (Harvey)

Constituents:

1. Water...................... 1000.0 cc.
2. Veal....................... 500.0 g.
3. Stomach digest solution.... 1000.0 cc.

Preparation:

(1) Mince finely fat-free veal.
(2) Add 500.0 g. to 1000.0 cc. water.
(3) Place 18 hours at 37°C.
(4) Pour the mixture onto a thick, clean cloth.
(5) Collect the fluid which drains thru the cloth together with that obtained by squeezing the meat in the cloth.
(6) Mix the fluid collected with an equal volume of stomach digest solution (see medium 998).
(7) Heat to 70°C.
(8) Make the reaction neutral to litmus.
(9) Add 7.0 cc. N/1 sodium hydroxide per liter.
(10) Filter thru well-wetted, thick filter paper.
(11) Dissolve 2.0% agar in (10).
(12) Steam 30 minutes
(13) Filter while hot thru well-wetted, thick filter paper.
(14) Distribute the filtrate into flasks.
(15) Add one of the added nutrients.

Sterilization: Method of sterilization not given.

Use: Cultivation of meningococci.

Added nutrients:

(a) Defibrinated blood. The blood was prepared and added as follows:
  (1) Add three parts distilled water to one part defibrinated blood.
  (2) Add 0.5 cc. of a 10.0% NaOH per 100.0 cc. (1).
  (3) Sterilize in the autoclave at 112°C. (The alkali prevents coagulation.)
  (4) Mix one part (3) with two parts basal agar at 80°C. The blood may be made just slightly alkaline to litmus by the addition of HCl before the addition to the agar.
(b) Serum.
  (1) Add 1.0 cc. formalin to 500.0 cc. horse serum.
  (2) Add 1.0% ammonia to (1) to neutralize to litmus.
  (3) Sterilize at 110°C. for 15 minutes.
  (4) Mix one part (3) with 3 parts basal agar.
(c) Serum (alkaline). Same as for (a) above, but use serum instead of blood.

Variants: Harvey used the basal medium without any additions.

Reference: Harvey (1921–22 pp. 74, 81, 99).

2041. Harvey's Serum Digest Agar

Constituents:

1. Distilled water.
2. Veal, fat free.
3. Minced stomach.
4. HCl.
5. Formalin.
6. Ammonia.
7. Serum.
8. Agar.

Preparation:

(1) Clean and wash a number of pigs' stomachs.
(2) Mince (1) finely.
(3) Add 10 cc. of strong hydrochloric acid to 1000 cc. water at 50°C.
(4) Add 200 g. (2) to (3) and keep at 50°C. for 20 hours.
(5) Raise the temperature to the boiling point.
(6) Pour the mixture on a thick clean cloth. Collect the fluid and that obtained by squeezing the cloth and its contents.
(7) Heat the fluid to 80°C. and make faintly alkaline to litmus while at 80°C.
(8) Filter thru well-wetted thick filter paper while hot.
(9) Steam 30 minutes.
(10) Filter again thru well-wetted thick filter paper.
(11) Mince finely, fat free veal, and add 500 g. to 1000 cc. water.
(12) Incubate for 18 hours at 37°C.
(13) Pour the mixture on a thick clean cloth.
(14) Collect the fluid, and that obtained by squeezing the cloth, and its contents.
(15) Mix (14) with an equal volume of pepsin digest solution (10).
(16) Heat to 70°C.
(17) Make the reaction neutral to litmus.
(18) Add 7 cc. of normal NaOH per liter.
(19) Filter thru well-wetted thick filter paper.
(20) Add sufficient agar to give a solid medium.
(21) Steam 30 minutes.
(22) Filter while hot thru well-wetted thick filter paper.
(23) Add 1 cc. formalin to 500 cc. horse serum.
(24) Add sufficient 1% ammonia solution to neutralize (23) to litmus.
(25) Mix one part (24) with two parts distilled water.
(26) Mix one part sterile (25) with three parts sterile (22).

Sterilization: Method of sterilization of (22) not specified. Sterilize (24) at 110°C. for 15 minutes in the autoclave.

Use: Isolation and cultivation of meningococcus.

Reference: Harvey (1921–22, p. 81).

2042. Harvey's Defibrinated Blood Digest Agar

Constituents:

1. Distilled Water.
2. Veal, fat free.
3. Minced stomach.
4. HCl.
5. Defibrinated blood.
6. NaOH.
7. Agar.

Preparation:

(1) Clean and wash a number of pigs' stomachs.
(2) Mince (1) finely.
(3) Add 10 cc. of strong hydrochloric acid to 1000 cc. water at 50°C.
(4) Add 200 g. (2) to (3) and keep at 50°C. for 20 hours.
(5) Raise the temperature to the boiling point.
(6) Pour the mixture on a thick clean cloth. Collect the fluid and that obtained by squeezing the cloth and its contents.
(7) Heat the fluid to 80°C. and make faintly alkaline to litmus while at 80°C.
(8) Filter thru well-wetted thick filter paper while hot.
(9) Steam 30 minutes.
(10) Filter again thru well-wetted thick filter paper.
(11) Mince finely, fat free veal, and add 500 g. to 1000 cc. water.
(12) Incubate for 18 hours at 37°C.
(13) Pour the mixture on a thick clean cloth.
(14) Collect the fluid, and that obtained by squeezing the cloth and its contents.
(15) Mix (14) with an equal volume of pepsin digest solution (10).
(16) Heat to 70°C.
(17) Make the reaction neutral to litmus.
(18) Add 7 cc. of normal NaOH per liter.
(19) Filter thru well-wetted thick filter paper.
(20) Add sufficient agar to give a solid medium.
(21) Steam 30 minutes.

(22) Filter while hot thru well-wetted thick filter paper.
(23) Mix one part defibrinated blood with 3 parts distilled water.
(24) Add 0.5 cc. of 10% NaOH per 100 cc. (23) to (23). (This permits sterilization in the autoclave without coagulation.)
(25) Mix one part sterile (24) with two parts sterile (22) under aseptic conditions.
(26) Slant.

Sterilization: Method of sterilization of (22) not specified. Sterilize (24) at 112°C. in the autoclave.

Use: Cultivation of meningococcus, etc.

Reference: Harvey (1921–22 p. 74).

2043. Martin's Digest Ascitic Fluid Agar

Constituents:

1. Martin's digest veal infusion agar	200.0 cc.
2. Ascitic fluid	100.0 cc.

Preparation:
(1) Mix 5.0 cc. of 10.0% NaOH and 100.0 cc. of ascitic fluid.
(2) Make sterile cool (1) faintly alkaline to litmus by the addition of 1–15 HCl.
(3) Mix one part sterile (2) at 80°C. with two parts sterile Martin's Digest Veal Infusion Agar (Harvey) (see medium 2040).

Sterilization: Sterilize (2) and (3) at 112°C. in the autoclave. Method of sterilization of agar not given.

Use: General culture medium.

Reference: Harvey (1921–22 p. 84).

2044. Harvey's Pepsinized Blood Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Blood, ox	500.0 g.
3. Pepsin powder	15.0 g.
4. Agar	

Preparation:
(1) Mix 500.0 cc. of ox blood, 15.0 g. powdered pepsin, 15.0 g. HCl and 1000.0 cc. distilled water.
(2) Keep for 12 hours at 40 to 42°C.
(3) Boil 30 minutes.
(4) Filter.
(5) Make faintly alkaline to litmus.
(6) Solidify with agar.

Sterilization: Not specified.

Use: General culture medium.

Reference: Harvey (1921–22 p. 77).

2045. Deycke and Voigtländer's Pepsinized Meat Agar

Constituents:

1. Distilled water	2000.0 cc.
2. Meat, horse	125.0 g.
3. HCl (50.0%)	2.0 cc.
4. Pepsin (Witte's)	3.0 g.
5. NaCl	8.0 g.
6. Glycerol	150.0 g.
7. Agar	

Preparation:
(1) Grind up 125.0 g. of lean horse meat with 3.0 g. of pepsin and 400.0 g. distilled water with 2.0 cc. of HCl.
(2) Place in an Erlenmeyer flask and incubate at 37°C. until there is complete solution.
(3) Dilute to 2 liters, neutralize with Na_2CO_3 and add 8.0 g. of NaCl.
(4) Add 150.0 g. glycerol and sufficient agar to solidify.

Sterilization: Method not given.

Use: Cultivation of parasitic and saprophytic forms.

Reference: Deycke and Vöigtlander (1901 p. 625).

2046. Kamen's Pepsinized Yeast Agar (Klimmer)

Constituents:

1. Water	1000.0 cc.
2. Yeast	
3. NaCl	3.0 g.
4. Agar	22.0 g.

Preparation:
(1) Autoclave 10,000.0 g. yeast with 20 liters water for 2 hours at 1.5 to 1 atmosphere pressure.
(2) Allow to settle and decant the liquid.
(3) Add pepsin and about 10 liters of 0.5% HCl solution to the thick yeast residue from (2).
(4) Incubate (3) for 5 days at 37°C.
(5) Allow to settle and decant the liquid or filter.
(6) Neutralize the liquid by the addition of NaOH.
(7) Add the liquid from (2) to (6).

(8) Heat at 50°C. until all the liquid has evaporated.
(9) Heat at 100 to 105°C. for one hour.
(10) Pound to a powder.
(11) Dissolve 6.0 g. (10), 3.0 g. of NaCl and 22.0 g. agar in a liter of water by heating in the steamer.
(12) Add 10.0% soda solution until the reaction is 0.05 to 0.07% alkaline.
(13) Boil for another hour in the steamer.
(14) Allow to stand over night at room temperature.
(15) Cut away the bottom of the agar containing the sediment
(16) Melt the clear agar and flask.

Sterilization: Sterilize on each of 3 successive days in the steamer for 1.5 hours.

Use: General culture medium.

Variant: Klimmer used 10.0 g. (10), 8.0 g. NaCl, 0.1 g. KNO_3, 22.0 g. agar and 0.2 g. Na_2CO_3 in a liter of water instead of the quantities given above.

Reference: Klimmer (1923 p. 171).

2047. Jensen's Milk Digest Agar

Same as medium 1112, but solidified with agar.

2048. Bacto Nutritive Caseinate Agar (Dehydrated)

Constituents:

1. Distilled water	1000.0 cc.
2. Peptonized milk Bacto	7.0 g.
3. Sodium caseinate	3.0 g.
4. Agar	12.0 g.

Preparation:
(1) Dissolve 22.0 g. of Bacto Nutritive Caseinate Agar (Dehydrated) in 1000.0 cc. distilled water by boiling.
(2) If sterilized at 15 pounds for 20 minutes pH = 6.5±.

Sterilization: Sterilize in the autoclave at 15 pounds pressure for 20 minutes.

Use: For differential and comparative milk counts.

Reference: Digestive Ferments Co. (1925 p. 13).

2049. Bacto Peptonized Milk Agar (Dehydrated)

Constituents:

1. Distilled water	1000.0 cc.
2. Peptonized milk	15.0 g.
3. Agar, Bacto	12.0 g.

Preparation:
(1) Dissolve 27.0 g. of Bacto Peptonized Milk Agar (Dehydrated) in 1000.0 cc. distilled water by boiling or autoclaving.
(2) Restore lost weight if necessary.
(3) If sterilized at 15 pounds pressure for 20 minutes pH = 6.5±.

Sterilization: Sterilize in the autoclave at 15 pounds pressure for 20 minutes.

Use: For bacteriological examination of dairy products.

Reference: Digestive Ferments Co. (1925 p. 14).

2050. Cole and Onslow's Trypsinized Casein Agar

Solidify medium 1130 with agar. The agar is added at step (18). (See medium 1130.)

2051. Teruuchi and Hida's Trypsinized Casein Agar

Constituents:

1. Water	1000.0 cc.
2. Casein	100.0 g.
3. Na_2CO_3 (10.0% soln.)	
4. Pancreatin	
5. NaCl (0.5%)	
6. Agar (2.0%)	

Preparation:
(1) Dissolve 100.0 g. of pure casein in 1 liter of 0.8% Na_2CO_3 (anhydrous).
(2) Add 5.0 to 10.0 g. of pancreatin (Gehe and Co.) and shake with chloroform.
(3) Place for 3 to 5 days in the incubator, shaking occasionally.
(4) Each day test the mixture for tryptophane, by adding a few drops of acetic acid to a sample and then adding bromine water.
(5) When the tryptophane content has reached its maximum and the tyrosine has separated out as white clumps, heat the fluid for a short time at 80°C.
(6) Filter and neutralize with a few cc. of HCl.
(7) Heat moderately, in a vacuum if possible, and evaporate to a syrupy thickness. (Possibly may be filtered again.)

(8) Place in a mortar and knead it with alcohol (amount not specified).
(9) Dry in a vacuum desiccator.
(10) Prepare a 2.0% agar solution in 0.5% HCl.
(11) To 90.0 cc. of (10) add 4 to 5.0 g. of (9) and exactly 10.0 cc. of a 10.0% Na_2CO_3 solution.
(12) Mix thoroly.
(13) Boil for several minutes.
(14) Pour immediately into Petri dishes.
(15) Allow the Petri dishes to remain uncovered until the agar has solidified.
(16) It is recommended to dry the agar plates in the incubator 1 to 2 hours before use.

Sterilization: Not specified.

Use: Isolation of cholera vibrio.

Reference: Teruuchi and Hida (1912 p. 573).

2052. Norris' Trypsinized Caseinogen Agar

Constituents:

1. Water	50,000.0	cc.
2. Na_2CO_3 commercial anhydrous	200.0	g.
3. Caseinogen	10.0	lbs.
4. Pancreatic extract	2,000.0	cc.
5. NaCl	125.0	g.
6. $CaCl_2$	6.25	g.
7. Agar	2,000.0	g.

Preparation:

(1) Boil 25 liters of water in a large tub of about 70 liters capacity.
(2) Add 2.
(3) Gradually shake in 3, and bring the whole to the boiling point.
(4) Add 25 liters of cold water.
(5) Reaction should be alkaline to litmus.
(6) If temperature is not more than 48°C. add 2 liters of pancreatic extract (prepared according to Cole, Lancet, July 1, 1916).
(7) Estimate amino acids by Sörensen's method.
(8) Digest (6) for 3.5 to 4 hours at 37°C.
(9) Then boil and filter thru muslin.
(10) Determine amount of amino-acids present again by Sörensen's method. This should show an increase equivalent to about 3.0 cc. N/10 NaOH per 10.0 cc. broth.
(11) Add 2.5 g. NaCl, 0.125 g. of $CaCl_2$ to each liter of the broth.
(12) Sterilize at 115°C. for one hour.
(13) Filter and place 100.0 cc. together with 4.0 g. desiccated agar (prepared according to Cunningham, Ind. J. Med. Res. April, 1919) into each round whiskey bottle.

Sterilization: Sterilize (13) at 120° for two hours.

Use: Substitute for meat in growth of organisms used for vaccines, *B. typhosus*, *B. para A* and *B*, and *B. cholerae*. The above quantities yield about 450 bottles.

Variants: Harvey prepared the medium as follows:

(1) Add gradually 100.0 g. commercial casein to 500.0 cc. boiling water containing 0.8% anhydrous sodium carbonate.

Note: Or double the quantity of washing soda.

(2) Raise the temperature to boiling point.
(3) Add 500.0 cc. cold water.
(4) Make the reaction faintly alkaline to litmus.
(5) Allow to cool to 45°C.
(6) Add 400.0 cc. pancreatic extract to the mixture.
(7) Keep 4 hours at 37°C.
(8) Raise the temperature to boiling point.
(9) Pour the digest mixture on to a wet, thick clean cloth.
(10) Collect the fluid which drains thru the cloth together with that obtained by squeezing the cloth.
(11) Filter the fluid collected thru well-wetted, thick filter paper.
(12) Bring the volume up to 1000.0 cc. by the addition of water.
(13) Add 2.5 g. sodium chloride and 0.125 g. calcium chloride.
(14) Adjust the reaction.
(15) Steam 30 minutes.
(16) Filter, while hot, thru well-wetted, thick filter paper.
(17) Sterilize in autoclave.
(18) Add to 30.0 cc. sterilized casein digest in large test tubes 4.0 g. desiccated agar.
(19) Sterilize in autoclave.

(20) Add the sterilized solution of agar in quantities of 30.0 cc. to casein digest already sterilized in quantities of 70.0 cc. in round quart bottles.

(21) Sterilize in autoclave.

Reference: Norris (1919–20 p. 541), Harvey (1921–22 p. 116).

2053. Norris' Simplified Caseinogen Agar

Constituents:

1. Tap water (or saline)	1000.0 cc.
2. Caseinogen (10.0%)	100.0 g.
3. Washing soda (0.8%)	8.0 g.
4. Agar	20.0 to 40.0 g.
5. Pancreatic extract (0.5%)	5.0 cc.

Preparation:

(1) Digest in an 0.8% aqueous solution of washing soda, 10.0% of caseinogen with 0.5% pancreatic extract at 37°C. for 24 hours.

(2) Concentrate the products of digestion on the water bath, yielding a paste of the consistency and color of Liebig's meat extract. This may be further dried in a desiccator over H_2SO_4 yielding a brittle resinous mass which may be powdered.

(3) To 1000.0 cc. of saline or tap water add 100.0 g. (10.0%) of (2) to 40.0 g. agar.

(4) Adjust reaction to +10. Indicator not specified.

Sterilization: Method not given.

Use: General culture medium. The author suggested the term "trypsinoids" for the digest (2). *B. typhosus* grew well on this medium. Brewer's yeast and fresh yeast were treated in like manner but caseinogen was the more nutritive of the two.

Reference: Norris (1919–20 p. 706).

2054. Teague and Deibert's Trypsinized Casein Agar

Constituents:

1. Water	100.0 cc.
2. Casein (10.0%)	10.0 g.
3. Trypsin	
4. Agar solution	

Preparation:

(1) Prepare a 10.0% casein solution and digest with trypsin at 37°C. for 8 days. Add chloroform to prevent bacterial growth.

(2) Boil (2) and filter thru cotton.

(3) Add various amounts of sterile (2) to an agar solution containing 20.0 g. agar and 5.0 g. NaCl per liter.

Sterilization: Sterilize (2) in the autoclave. Sterilization of agar or final sterilization not specified.

Use: To study growth requirements of Unna-Ducrey bacillus. Authors reported that the medium did not support growth.

Reference: Teague and Deibert (1922 p. 70).

2055. Sierakowski's Trypsinized Casein Lactose Agar

Constituents:

1. Water	1000.0 cc.
2. Trypsinized casein (10.0%)	100.0 g.
3. Lactose (1.0%)	10.0 g.
4. Brom thymol blue	0.3 g.
5. Agar (2.0%)	20.0 g.

Preparation:

(1) Prepare a tryptic digest of casein and adjust to pH = 7.0. Method of preparation not given.

(2) Dissolve 10.0% (1), 1.0% lactose, 2.0% agar and 0.3 parts per 1000 brom thymol blue in water.

Sterilization: Not specified.

Use: Isolation of colon-typhoid group. Author reported that *B. coli* and lactose fermenters gave yellow colonies. Typhoid and non-lactose fermenters gave blue colonies. Staphylococci and other organisms resisting the gram stain were inhibited.

Reference: Sierakowski (1923 p. 1003).

2056. Harvey's Trypsinized Caseinogen Blood Agar

Constituents:

1. Distilled water	100.0 cc.
2. Blood (human or rabbit)	20.0 cc.
3. Trypsinized caseinogen agar	400.0 cc.

Preparation:

(1) Add 20.0 cc. sterile human or rabbit blood to 100.0 cc. distilled water in a flask furnished with a rubber cork and containing glass beads.

(2) Shake vigorously to prevent formation of large fibrin masses.

(3) Add 5.0 cc. pancreatic extract, 5.0 cc. enterokinase solution, and 1.5 cc. chloroform.
(4) Shake to mix.
(5) Incubate 8 days with daily shaking the first three days.
(6) Remove the flask without disturbing the sediment.
(7) Pipette off for use, with sterile precautions, as much clear supernatant fluid as possible.
(8) Filter the remainder thru sterilized filter paper.
(9) Mix one part sterile (9) with four parts sterile trypsinized caseinogen agar (see Harvey's variant of medium 2052) at 45°C.

Sterilization: Method of sterilization of agar not given.

Use: Cultivation of *B. influenzae*.

Reference: Harvey (1921–22 p. 117).

2057. Harvey's Blood Tryptamine Agar

Constituents:

1. Distilled water	3000.0 cc.
2. Casein	100.0 g.
3. Agar	60.0 g.
4. Blood, defibrinated	150.0 cc.

Preparation:

(1) Prepare a suspension of one part casein in 10 parts water in a well stoppered bottle.
(2) Shake well to break up clumps.
(3) Adjust the reaction if necessary with the help of cresol red.

NOTE: The optimum reaction for the tryptic digestion of casein is about pH = 8.1 at which point cresol red indicator solution gives a reddish violet color and phenolphthalein remains colorless.

(4) Add per liter 60.0 cc. pancreatic extract and 5.0 cc. toluol.
(5) Shake to mix.
(6) Digest at 39°C. 10 days, with daily shaking and addition of more toluol if necessary.
(7) Add per liter, 100.0 cc. 7.5% hydrochloric acid.
(8) Steam 20 minutes.
(9) Filter thru well-wetted, thick, filter paper.
(10) Make the reaction nearly neutral to litmus with 5.0% sodium hydroxide.
(11) Mix one part (10) and two parts water.
(12) Make distinctly acid to litmus by the cautious addition of strong hydrochloric acid.
(13) Add agar 2.0%.
(14) Steam 150 minutes.
(15) Filter, while hot, thru well-wetted, thick, filter paper by placing filter funnel, stand, and receptacle for filtrate in the steamer and steaming till filtration is completed.
(16) Add per liter of melted agar at 65°C., 50.0 cc. defibrinated sheep blood and the beaten up whites of 2 eggs.
(17) Steam 50 minutes.
(18) Strain the mixture thru a fine wire sieve and squeeze the fluid out of the clot.
(19) Filter thru glass wool, taking care to keep the mixture hot during the process.
(20) Adjust the reaction.
(21) Distribute in test tubes.

Sterilization: Sterilize 15 minutes at 100°C. on three successive days.

Use: General culture medium.

Reference: Harvey (1921–22 p. 74).

2058. Teague and Deibert's Serum Trypsinized Casein Agar

Constituents:

1. Water	100.0 cc.
2. Casein (10.0%)	10.0 g.
3. Trypsin	
4. Serum (Rabbit or sheep)	
5. Agar solution	

Preparation:

(1) Prepare a 10.0% casein solution and digest with trypsin at 37°C. for 8 days. Add chloroform to prevent bacterial growth
(2) Boil and filter thru cotton.
(3) Obtain sheep or rabbit serum from freshly drawn defibrinated blood.
(4) Heat (3) for 15 minutes at 55°C.
(5) Add various amounts of sterile (3), to an agar solution containing 20.0 g. agar, and 5.0 g. NaCl per liter.

Sterilization: Sterilize (2) in the autoclave. Sterilization of agar, or final sterilization, not specified.

Use: To study growth requirements of Unna-Ducrey bacillus. Authors reported that sheep serum medium gave no growth. Remaining media gave good or excellent growth.

Variants: The author added various amounts of the following blood cell infusion to the medium prepared as given above:

1. (1) Add 2.0 cc. of red blood cells (obtained by centrifuging defibrinated blood (rabbit) and removing the serum) to 10.0 cc. of normal NaCl solution.
2. (2) Keep the temperature at 100°C. for 3 minutes.
3. (3) Shake the tubes and allow to cool.
4. (4) Centrifuge and obtain the supernatant fluid.

Reference: Teague and Deibert (1922 p. 70).

2059. Kulp and Rettger's Trypsinized Casein Agar

Constituents:

1. Water	2000.0 cc.
2. Na_2CO_3 (1.0%)	20.0 g.
3. Casein	200.0 g.
4. Trypsin (Fairchild's)	3.0 g.
5. Galactose (1.0%)	20.0 g.
6. Meat extract	6.0 g.
7. Agar	to solidify

Preparation:

(1) Prepare a 1.0% solution of Na_2CO_3 and heat to boiling.

(2) Add 20.0 g. of granulated casein to 2 liters of boiling (1). Add the casein in small quantities at a time, and stir after each addition until complete solution has taken place.

(3) Cool to about 40°C.

(4) Add 10 or 12.0 cc. water containing 3.0 g. of fresh Fairchild's trypsin.

(5) Mix well and add 25.0 cc. chloroform.

(6) Flask and incubate at 37°C. for 48 hours.

(7) Neutralize to litmus by the addition of HCl.

(8) Heat in a double boiler to remove the chloroform.

(9) Filter. If chemically pure casein is used the filtrate will be clear and have a bright amber color. Commercial casein makes a darker colored product due to the small lactose content.

(10) Distribute in quantities equal to 10.0 g. of the original casein into flasks.

(11) When ready for use add water to make 1000.0 cc. to each sterile flask and dissolve 3.0 g. commercial meat extract, agar to give the desired consistency, and 1.0% galactose in each flask.

Sterilization: Sterilize (10) in the autoclave. Final sterilization not specified.

Use: Cultivation of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*. The authors reported that any other carbohydrate might be used instead of galactose, but galactose favored the growth of the organisms most. This medium is slightly inferior to a medium prepared from klim powdered skim milk. See the following medium.

Variants: Any other carbohydrate may be used instead of galactose.

Reference: Kulp and Rettger (1924 p. 363).

2060. Kulp and Rettger's Trypsinized Klim Agar

Constituents:

1. Water	2000.0 cc.
2. Na_2CO_3 (1.0%)	20.0 g.
3. Klim (powdered skim milk)	200.0 g.
4. Meat extract	3.0 g.
5. Trypsin (Fairchild's)	3.0 g.
6. Galactose (0.5%)	10.0 g.
7. Agar to solidify	

Preparation:

(1) Prepare a 1.0% solution of Na_2CO_3.

(2) Add 200.0 g. of klim (powdered skim milk) to 2000.0 cc. of cold (1). Add the klim in small quantities and stir after each addition, until solution is complete.

(3) Heat to about 40°C.

(4) Add 10.0 to 12.0 cc. of water containing 3.0 g. of fresh Fairchild's trypsin.

(5) Mix thoroly and add 25.0 cc. chloroform.

(6) Flask and incubate at 37°C. for 48 hours.

(7) Add HCl to neutralize to litmus.

(8) Heat in a double boiler to remove the chloroform.

(9) Distribute into 100.0 cc. lots.

(10) When ready for use add water to each sterile flask to make 1000.0 cc.,

agar to give the desired consistency and 0.5% galactose.

Sterilization: Sterilize (9) in the autoclave. Final sterilization not given.

Use: Cultivation of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*. The authors reported that any other carbohydrate might be used instead of galactose, but galactose favored the growth of the organisms most. This medium is slightly superior to preceding casein digest medium.

Variants: Any other carbohydrate may be used instead of galactose. The carbohydrate may be used in 1.0% instead of 0.5% concentration if desired.

Reference: Kulp and Rettger (1924 p. 363).

2061. Harvey's Trypsinized Ox Heart Agar

Constituents:

1. Water	400.0 cc.
2. Heart, ox	
3. NaCl	1.0 g.
4. $CaCl_2$	0.5 g.
5. Blood (citrated laked)	10.0 cc.

Preparation:

(1) Mince finely an averaged sized ox heart.
(2) Add to 400.0 cc. tap water.
(3) Heat slowly to 75°C.
(4) Allow to cool to 45°C.
(5) Add trypsin solution to 1.0%.
Note: e.g. Liq. trypsin Co. (A and H).
(6) Place in incubator 150 minutes.
(7) Test for peptone by the Biuret test.
Note: Add 1.0 cc. 5.0% copper sulphate to 5.0 cc. trypsin digest, followed by 5.0 cc. N/1 potassium hydroxide. Note the color change. If the color is pink, peptonization is complete, if bluish purple incomplete.
(8) Make faintly acid to litmus with 4.0% acetic acid.
(9) Boil 15 minutes.
(10) Allow the solid matter to settle.
Note: Or simply strain thru cloth.
(11) Pour off the supernatant fluid.
(12) Add: sodium chloride 1.0 g., calcium chloride 0.5 g.
(13) Make faintly alkaline to litmus.
(14) Steam 45 minutes.
(15) Bring up to original volume.
(16) Adjust the reaction.
(17) Steam 30 minutes.
(18) Clarify and filter.
(19) Bleed a rabbit directly from the carotid into 1.5% sodium citrate solution.
(20) Dilute with 0.85% sterile salt solution to give a 5.0% suspension of blood.
(21) Add 10.0% ether.
(22) Shake to mix.
(23) Leave the sediment 24 hours.
(24) Draw off the laked blood into a sterile bottle.
(25) Add an excess of ether.

Sterilization: Final sterilization not specified.

Use: Cultivation of highly pathogenic organisms.

Variants: The author cultivated meningococci in a medium prepared as follows:

(1) Steam 50.0 g. pea flour and 100.0 g. NaCl in 1000.0 cc. water for 30 minutes, with occasional stirring.
(2) Filter thru thick filter paper.
(3) Sterilize (method not given).
(4) Mix 5.0% of (3) with the agar obtained at step (18) in the medium given above. 25.0% rabbit or horse serum may be added if desired.

Reference: Harvey (1921–22 pp. 75, 77, 114, 119).

2062. Norris' Trypsinized Mutton Agar

Constituents:

1. Water	2000.0 cc.
2. Mutton, fat-free	2.0 lbs.
3. NaCl	5.0 g.
4. $CaCl_2$	0.25 g.
5. Agar	80.0 g.

Preparation:

(1) Mince two pounds fat free mutton.
(2) Place 2 liters of water and (1) in a 3 liter pot.
(3) Autoclave at 130°C. for one hour.
(4) Keep over night, but if urgently wanted, cool to 45°C. and continue as below.
(5) Test amino acidity by Sörensen's method.
(6) Add 40.0 cc. pancreatic extract per liter.
(7) Digest four hours at 37°C.
(8) Retest amino acid acidity.

(9) Filter thru muslin to remove fat.
(10) Add 1.0 cc. glacial acetic acid per pot.
(11) Sterilize by heating up to 110°C. and then cool.
(12) Filter to remove mince.
(13) Make slightly alkaline to litmus and then add 2.5 g. NaCl and 0.125 g. $CaCl_2$ per liter.
(14) Sterilize at 115°C. for one hour.
(15) Filter thru paper.
(16) Put 100.0 cc. broth into each whiskey bottle.
(17) Sterilize at 120°C. for one hour.
(18) Add 4.0 g. desiccated agar to each bottle.

Sterilization: Sterilize (18) at 120°C. for one hour.

Use: Growth of organisms for producing vaccines. The quantities employed yielded about 15 or 16 bottles. Authors reported this medium inferior to medium 2052 for vaccine production.

Reference: Norris (1919–20 p. 542).

2063. Harvey and Iyengar's Desiccated Tryptonized Mutton Bouillon Agar

Constituents:

1. Trypsinized mutton........	1000.0 cc.
2. Agar	

Preparation:

(1) Prepare a tryptic digest of mutton bouillon (method not given).
(2) Adjust (1) to pH = 8.0.
(3) Cut up agar fiber into small pieces.
(4) Add (3) to (2). (6.0% by weight.)
(5) Autoclave for one hour at 120° to melt the agar.
(6) Filter thru cotton, wool and muslin into a tin receptacle.
(7) Cut the agar out of the receptacle and into slices.
(8) Pass slices thru meat mincing machine with a finely perforated outlet disc.
(9) Spread minced nutrient agar on metal or other type of trays.
(10) Dry in hot air oven or any other convenient way.
(11) Store powder in sterile glass stoppered bottle.
(12) Add 4.0% to 6.0% by weight of (11) to water in test tubes or flasks.

Sterilization: Keep (11) immersed for 1 to 2 hours in boiling water or in a steam sterilizer to bring the medium into solution and to sterilize.

Use: Desiccated nutrient medium.

Variants: Vardon prepared a similar medium as follows:

(1) Wash a quantity of agar fiber in tap water containing 0.25% acetic acid.
(2) Wash in several changes of tap water until the washings are neutral to litmus paper.
(3) Place the washed fiber on perforated or wire gauze trays and cover with thin muslin.
(4) Expose the agar in the trays to the sun to dry.
(5) Prepare a double strength[1] (composition not given) tryptic digest of mutton.
(6) Filter thru paper and measure the filtrate.
(7) Adjust the reaction of the filtrate to pH = 8.0.
(8) Add[2] after this adjustment 25.0% more alkali of the strength used.
(9) Add 9.0%[3] by weight of washed and dried agar fiber.
(10) Place the mixture for one hour in the autoclave, at 120°C. to thoroly dissolve the agar.
(11) Allow the nutrient agar to solidify[4] in a metal funnel.
(12) Turn out the clear solidified mass and cut into pieces.
(13) Pass thru a meat mincing machine with finely perforated outer disc.
(14) Spread the minced agar on metal trays.
(15) Dry in hot air oven at a temperature not exceeding[5] 60°C.
(16) Remove as soon as the agar is thoroly dried.
(17) Scrape the dried material off the trays and grind to powder.
(18) Replace the powder in the hot oven and complete the drying process.
(19) Record the final[6] weight of the powder.
(20) Store in tight stoppered bottles.

NOTES: [1]All the constituents of the ordinary tryptic digest bouillon (Douglas' medium) are doubled, i.e. the meat, the liquid pancreatic extract, the sod. chloride, and the calcium chloride. [2]This addition was found by trial to be necessary in order to give a final medium of pH = 7.0 to 7.2. Variations in the mode of manufacture from that here given would demand trials to determine the exact amount of additional alkali required. [3]Found to be the requisite amount by trial. [4]By keeping the agar at a temperature at which it remains fluid and allowing time for sedimentation, undissolved matter may be collected at the extremity of the cone. This extremity may be cut off and rejected after the agar is allowed to solidify. In practice the undissolved material may be left in the agar, for it collects untimately in very small amount in the butt of the sloped agar medium and does not at all inferfere with its transparency. [5]At higher temperatures the medium is apt to acquire a permanently brown coloration. [6]On this weight taken in conjunction with the volume of nutrient bouillon converted into agar is based the calculation of the amount of water which it is necessary to add to the powder in order to give the medium required for use.

(21) Weigh out a quantity of dried powder sufficient for the purpose in hand.

NOTE: This may be an amount to make a single test tube of medium or several tubes. If the requisite amount is weighed out with precaution into each test tube separately, or roughly measured out, the sterilization required, if the test tubes are themselves sterile, need not under ordinary circumstances be more than is required to dissolve the agar in the water. As an example of the amount of powder required we may take it that, say 1,600.0 cc. of filtrate was obtained from 2,000.0 cc. of unfiltered bouillon and that in dried powder this represented 128.0 g. Then, we recollect that the original bouillon is double, strength, it would require an addition of 1,600.0 cc. water to 64.0 g. of powder to produce a single strength medium. As a percentage this would be given as 4.0% of powder to be added to the water.

(22) Add the requisite amount of water.

(23) Place in an autoclave for 30 minutes at 120°C. to dissolve the powder.

(24) Distribute the liquid nutrient agar in test tubes in amounts of 7 to 10.0 cc.

(25) Sterilize in the autoclave 30 minutes at 120°C. or in the steamer for 20 minutes on three successive days.

(26) Slope the tubes.

References: Harvey and Iyengar (1921–22 p. 365), Vardon (1923–24 p. 429).

2064. Harvey and Iyengar's Desiccated Bile Salt Trypsinized Mutton Agar

Constituents:

1. Tryptic digest of mutton..............	1000.0 cc.
2. Agar.................	60.0 g.
3. Sodium taurocholate.	5.0 g.
4. Neutral red (1.0%)...	2.0 to 5.0 cc.
5. Lactose..............	10.0 g.

Preparation:

(1) Prepare a tryptic digest of mutton.

(2) Adjust (1) to pH = 8.0.

(3) Cut up agar fiber into small pieces.

(4) Add (3) to (2). (6.0% by weight.)

(5) Autoclave at 120° for an hour to melt the agar.

(6) Filter thru cotton, wool and muslin into a tin receptacle.

(7) Cut the agar out of the receptacle and into slices.

(8) Pass slices thru meat mincing machine with a finely perforated outlet disc.

(9) Spread minced nutrient agar on metal or other type of trays.

(10) Dry in hot air oven or any other convenient way.

(11) Store powder in sterile glass stoppered bottle.

(12) Dissolve 40.0 g. of (11) and 5.0 g. of 3 in 1000.0 cc. distilled water by heating in the steamer.

(13) Prepare fresh 1.0% solution of neutral red.

(14) Add to (12) 0.2% to 0.5% of (13) and 1.0% lactose. Dissolve the lactose by gentle heating. (Lactose

may be added in the form of a strong sterilized solution and added to the medium after sterilization.)

Sterilization: Sterilize 20 minutes at 100°C. on each of 3 successive days.

Use: Desiccated medium for colon typhoid group.

Reference: Harvey and Iyengar (1921–22 p. 366).

2065. Vardon's Desiccated Aronson's Agar

Constituents:

1. Tryptic digest of mutton	1000.0 cc.
2. Agar (9.0%)	9.0 g.
3. Na_2CO_3 (10.0% anhydrous solution)	12.0 cc.
4. Sucrose (20.0% solution)	10.0 cc.
5. Dextrin (20.0% solution)	10.0 cc.
6. Fuchsin, basic (sat. alcoholic solution)	0.8 cc.

Preparation:

(1) Add 9.0% washed fiber agar to 1000.0 cc. of double strength tryptic digest of mutton. (See variant of medium 1715.)

(2) Heat for 60 minutes at 120°C. to dissolve the fiber agar.

(3) Add 12.0 cc. 10.0% anhydrous sodium carbonate to the nutrient agar.

(4) Steam 15 minutes at 100°C.

(5) Add while hot 10.0 cc. 20.0% sucrose, 10.0 cc. 20.0% dextrin, and 0.8 cc. saturated alcoholic solution of basic fuchsin.

(6) Allow to solidify.

(7) Cut up the solidified medium and pass thru a meat-mincing machine with small outlet holes.

(8) Spread the minced medium on metal trays.

(9) Dry at a temperature not exceeding 60°C.

(10) Grind the medium when dried to a powder.

(11) Weigh the powder obtained.

(12) Complete the drying at a temperature not exceeding 60°C.

(13) Record the final weight.

(14) Add 100.0 cc. water to the requisite amount of (13). (See step (21) of variant of medium 2063.)

(15) Dissolve the powder by steaming 15 minutes at 110°C.

(16) Add, while liquid, 2.0 cc. 10.0% freshly prepared sodium sulphite.

(17) Pour plates or prepare slopes.

Sterilization: See step (15) above.

Use: Desiccated culture medium.

Reference: Vardon (1923–24 p. 432).

2066. Deycke and Voigtländer's Tryptic Meat Agar

Constituents:

1. Water	3000.0 cc.
2. Glycerol	190.0 g.
3. Pancreas	
4. Meat	200.0 g.
5. NaOH (3.0%)	300.0 cc.
6. Agar to solidify	

Preparation:

(1) Allow the finely chopped pancreas of hogs to remain on ice for 24 hours.

(2) Mix with 40.0 g. glycerol and 160.0 cc. water.

(3) Press out the fluid from this mixture.

(4) Dissolve 200.0 g. meat in 300.0 cc. of 3.0% NaOH solution.

(5) Filter (4) and neutralize.

(6) Add 0.25% Na_2CO_3 (siccum) to (5).

(7) Sterilize (method not given).

(8) Add 50.0 g. of (3) to (7).

(9) Incubate for 7 to 10 hours at 37°C.

(10) Neutralize with HCl.

(11) Dilute with water to 3 liters.

(12) Add 150.0 g. glycerol and the usual amount of agar to (11).

Sterilization: Not specified.

Use: Cultivation of parasitic and saprophytic forms. Authors reported that it was necessary to add glucose to cultivate streptococci. Reitz used this medium in the bacteriological examination of butter for the detection of diphtheria bacilli.

References: Deycke and Voigtländer (1901 p. 626), Reitz (1906 p. 723).

2067. Murray and Ayrton's Heart Infusion Agar

Constituents:

1. Distilled water	2000.0 cc.
2. Agar	40.0 g.
3. Heart, ox	1000.0 g.
4. NaCl (0.25%)	2.5 g.
5. KCl (0.01%)	0.2 g.
6. $CaCl_2$	0.1 g.

Preparation:

(1) Mince fresh fat free ox heart muscle and suspend in twice its weight of distilled water in an open can in a steamer.
(2) Raise the temperature gradually to 70°–75°C. and hold for three hours.
(3) Raise the temperature to 100°C. and keep at this temperature for 15 minutes.
(4) Pour the infusion and meat on a wetted butter muslin and filter until clear.
(5) This constitutes the stock infusion and may be sterilized or used immediately to prepare a 4.0% agar solution.
(6) Prepare an infusion as above, steps (1) and (2).
(7) Filter until clear thru the meat particles suspended on well wetted butter muslin.
(8) Prepare an equal volume of 4.0% agar solution in the stock infusion (5).
(9) Cool (8) to 70°C. and mix with (7).
(10) Heat in the steamer for one hour.
(11) Allow to set and stand over night.
(12) Melt the following morning and strain through lint.
(13) Suspend the residual meat and fine coagulum from (4) and (7) in a quantity of N/100 HCl equal to the weight of the original raw meat.
(14) Raise to 100°C. and autoclave at 130°C. (25 lbs. pressure) for 30 minutes.
(15) Allow to cool to 37°C., incubate 24 hours, to test sterility.
(16) Add 2.0% of sterile pancreatic extract and incubate for 5 to 15 hours.
(17) Add 0.8% anhydrous Na_2CO_3 in the form of a sterile 32.0% solution.
(18) Allow digestion to continue until a Sorensen figure of not less than 20 is produced. (Titrate the digest in the presence of neutralized formalin, using phenolphthalein as an indicator, and express the result in cc. of N/10 NaOH required to neutralize the amino acids in 10.0 cc. of the filtered digest.)
(19) Add 2.0% of N/10 HCl and autoclave.
(20) Filter while hot through paper.
(21) Add 0.25% NaCl, 0.02% KCl, 0.01% $CaCl_2$ and the desired amount of (20) to (12).
(22) Adjust to pH = 7.2.
(23) Tube.

Sterilization: Autoclave at 120°C. for 20 minutes.

Use: Cultivation of meningococci.

Reference: Murray and Ayrton (1924–25 p. 49).

2068. Hottinger's Agar (Park, Williams and Krumwiede)

Constituents:

1. Water	1500.0 cc.
2. Meat	750.0 g.
3. Na_2CO_3	3.0 g.
4. Agar	1.5%

Preparation:

(1) Free meat from fascia and cut in finger thick pieces.
(2) Heat 1500.0 cc. of water to boiling.
(3) Drop 750.0 g. of (1) into (2), piece by piece, stirring constantly.
(4) Boil strongly and remove from the fire.
(5) Remove the meat and run thru a chopping machine.
(6) Cool the water to 37°C. and add 1.5 g. Na_2CO_3 per liter.
(7) Put the chopped meat in 2 liter Erlenmeyer flasks, 550.0 g. per flask.
(8) Add (6) to each flask, filling the flask to the neck.
(9) Add 3.0 g. pancreatin, 10.0 cc. chloroform and 10.0 cc. toluol to each flask.
(10) Cork tightly and shake well.
(11) Incubate at 37°C. over night.
(12) Shake the next day and add more pancreatin unless the fluid shows a yellow color and particles of meat look smaller.
(13) Continue digestion for four or five days at room temperature, or for two or three days in the incubator. The meat should be finely divided at the end of this time.
(14) Decant the liquid thru cheese cloth.
(15) Add an equal volume of water to the residue, shake well and again decant.
(16) Place the meat on cheese cloth and allow to drain.
(17) Boil the filtrate for a few minutes.

(18) Filter thru absorbent cotton and paper until clear.
(19) Mix the filtrate with an equal amount of water.
(20) Solidify (19) by the addition of 1.5% agar in the usual manner.

Sterilization: Sterilize at 15 pounds pressure for 30 minutes.

Use: General culture medium.

Reference: Park, Williams and Krumwiede (1924 p. 119).

2069. Gordon and Hines' Trypagar

Constituents:

1. Water	
2. Pea flour	100.0 g.
3. NaCl	100.0 g.
4. Heart bullock	500.0 g.
5. Agar	20.0 g.
6. $CaCl_2$	0.125 g.

Preparation:

(1) Add a liter of water to a 100.0 g. of pea flour and 100.0 g. NaCl.
(2) Mix thoroly and steam for 30 minutes, stirring occasionally.
(3) Allow to settle and filter the supernatant liquid.
(4) Add 1000.0 cc. of water to 500.0 g. of finely chopped bullock heart and make slightly alkaline to litmus by the addition of 20.0% KOH solution.
(5) Heat (4) slowly at 75 to 80°C. for 5 minutes.
(6) Cool to 37°C. and add 1.0% liquor trypsinae (Allen and Hanbury's) and incubate at 37°C. for two and one-half to three hours.
(7) Test for peptone using the Biuret test.
(8) Render slightly acid to litmus by the addition of glacial acetic acid and boil for 15 minutes.
(9) Leave over night in a cool place and siphon off the clear liquid in the morning.
(10) Make faintly alkaline to litmus.
(11) Weigh out 20.0 g. agar and cut in fine pieces with a scissors.
(12) Wash (11) with water and drain thoroly. Add a sufficient quantity of water to cover the agar and add 2.5 g. of glacial acetic acid per liter of water.
(13) Mix acid and agar thoroly and leave for 15 minutes.
(14) Pour off the water and wash thoroly until the agar is free from acetic acid.
(15) Add the agar obtained from (14) to 1000.0 cc. of (10).
(16) Add 0.125 g. $CaCl_2$.
(17) Autoclave for 45 minutes to dissolve the agar.
(18) Neutralize to phenolphthalein by the addition of N/10 KOH while hot.
(19) Cool to 60°C. and add the white of 2 eggs beaten up with the crushed shells.
(20) Autoclave again at 118°C. for 75 minutes or heat in the steamer for 2 hours.
(21) Filter.
(22) Add 2.0% of sterile (3).
(23) Distribute as desired.

Sterilization: Sterilize (23) in the ordinary way.

Use: Cultivation of meningococci. Wood used a similar medium for the cultivation of diphtheria bacilli.

Variants: Wood added 0.3 cc. of a sterile 1.0% telluric acid solution to 10.0 cc. of the agar for the cultivation of diphtheria bacilli.

Reference: Gordon and Hines (1916 p. 682), Wood (1921 p. 562).

2070. Duval and Harris' Tryptic Digest Agar

Constituents:

1. Water	1000.0 cc.
2. Agar (3.0%)	30.0 g.
3. Tryptic Digest Solution	1000.0 cc.

Preparation:

(1) Prepare a 3.0% agar solution in water.
(2) Tube.
(3) Melt sterile tube of (2) and cool to 45°C.
(4) Mix equal parts of the sterile agar with Duval and Harris' Tryptic Digest Solution (See medium 1134).
(5) Slant and cool.

Sterilization: Method of sterilization of agar solution not given. The Tryptic Digest Solution is sterilized by filtering thru a Berkefeld filter.

Use: Cultivation of leprosy bacillus.

Variants: If desired, glycerol, peptone, salt, etc., may be added in the preparation of this agar.

Reference: Duval and Harris (1911 p. 169).

2071. Distaso's Trypsinized Serum Agar

Constituents:

1. Water	1500.0 cc.
2. Serum (beef or sheep)	500.0 cc.
3. Agar	30.0 to 40.0 g.

Preparation:

(1) Mix equal parts (500.0 cc.) of water and sheep or beef serum.
(2) Sterilize at 120°C. for 15 minutes.
(3) Digest for 24 hours at 60°C. with a pancreatic extract from a hog in the presence of chloroform. Activate the extract with an extract of the upper portions of the small intestine.
(4) Filter on paper.
(5) Add 30.0 or 40.0 g. of agar to 1000.0 cc. of water.
(6) Sterilize (5). (Method not given.)
(7) Mix equal parts (4) and (6).
(8) Tube.

Sterilization: Method of final sterilization not given.

Use: Culture medium for tubercle bacilli, strict anaerobes, *B. proteus* and others.

Reference: Distaso (1916 p. 600).

2072. Stickel and Meyers' Trypsinized Blood Clot Agar

Constituents:

1. Tap water	1000.0 cc.
2. Blood clots	500.0 g.
3. K_2HPO_4	2.0 g.
4. Pancreatic extract	10.0 g.
5. Agar	20.0 g.

Preparation:

(1) Obtain 10 liters fresh beef blood from the abbatoir.
(2) Decant and store the serum (which has separated on standing) in a refrigerator.
(3) Weigh the blood clots and mix 500.0 g. with 1 liter tap water.
(4) Place the mixture in an enameled pot, bring slowly to a boil and boil slowly for 5 minutes stirring constantly.
(5) Strain fluid thru cheese cloth and pass the residue thru a fruit press, cool to 37°C.
(6) Make the thick brownish fluid slightly alkaline to litmus.
(7) Add 1.0% pancreatic extract and incubate at 37°C. for 5–24 or 48 hours.
(8) When the process is sufficiently advanced, render slightly acid with glacial acetic acid and boil slowly 15 minutes.
(9) Either filter or decant the clear fluid which results on placing the digest over night in a cool place.
(10) Adjust the reaction as desired.
(11) Dissolve 3 and 5 in (10).
(12) Adjust to desired reaction using litmus or preferably to a definite H-ion concentration (pH = 7.0 to 7.5).
(13) Clear (12) by adding 5–10.0% of the decanted beef serum. Steam 45 to 60 minutes.
(14) Remove (13) from steamer and allow the clot to form as a compact mass. Decant or better centrifuge the medium to remove it.

Sterilization: Sterilize at 100° for 30 minutes on two successive days.

Use: General inexpensive culture medium. Authors reported that this medium was excellent for primary isolation of highly parasitic organisms.

Reference: Stickel and Meyer (1918 p. 81).

2073. Stickel and Meyer's Tryptic and Peptic Digest Agar

Constituents:

1. Tap water	4000.0 cc.
2. Pig's stomach (minced)	400.0 g.
3. Beef, liver, placenta, blood clots (minced)	400.0 g.
4. HCl (Baker Chemical Co.)	40.0 g.
5. Pancreatic extract or "Bacto" trypsin	40.0 g.
6. K_2HPO_4	8.0 g.
7. Agar	80.0 g.

Preparation:

(1) Wash clean and mince fine 5 or more large pigs stomachs. Mince an equal amount of clean pig or beef liver, cheap fat-free beef, placenta or blood clots.
(2) Mix 2, one of 3, and 4 at 50°C. and keep at 50°C. for 18 to 24 hours.
(3) Make a biuret and tryptophane test. When both are + the digest is yel-

lowish green and contains very little undigested debris.

(4) Transfer to large bottles and steam for 10 minutes to stop digestion.

(5) Cool to 80°C. and make faintly alkaline to litmus using 2N KOH or 2N Na_2CO_3.

(6) Cool to 37°C. and add 1.0% pancreatic extract or "Bacto" trypsin.

(7) Keep the mixture at 37°C. for 3 to 10 hours depending on the action of the trypsin and the digestion desired. Control the process by repeated tests for tryptophane.

(8) When trypsinizing is sufficiently advanced render slightly acid with glacial acetic acid, and bring slowly to boiling point for 10 minutes.

(9) Filter thru paper or keep in cool place over night and decant the clear liquid in the morning.

(10) Add agar and K_2HPO_4 and autoclave at 10 pounds pressure for 45 minutes or heat in double boiler to 100°C. until the agar is dissolved.

(11) Restore the volume lost by evaporation.

(12) Adjust to slightly alkaline to litmus or pH = 7.3 by using 2N KOH or NaOH.

(13) Cool to 60°C. and add white of egg with crushed shells or for sake of economy, ordinary beef or sheep serum, in the quantity of 25–50.0 cc. per liter.

(14) Autoclave for one hour at 115°C.

(15) Filter thru cotton and distribute in 200–500.0 cc. quantities.

Sterilization: Sterilize at 100° for 30 minutes on two successive days or at 10 pounds pressure for 15 minutes.

Use: General inexpensive culture medium.

Variants: The authors prepared a sugar-free medium as follows:

(1) Carry out procedure as above steps (1) thru (5).

(6) Cool to 37°C. and add 1.0% pancreatic extract or Bacto trypsin, 8.0 g. K_2HPO_4 and 1.0% of a culture of *B. saccharolyte.*

(7) Keep the mixture at 37°C. for 3 to 10 hours depending on the action of the trypsin and digestion desired. Control the process by repeated tests for tryptophane.

(8) When the digest is sugar free add 4.0 g. $CaCO_3$ and 80.0 g. agar.

(9) Autoclave at 10 pounds pressure for 45 minutes or heat in double boiler to 100°C. until the agar is dissolved. Remainder of the preparation same as medium given above.

Reference: Stickel and Meyer (1918 p. 80).

2074. Deycke and Voigtländer's Tryptic and Peptic Meat Digest Agar

Constituents:

1. Distilled water to	1950.0 cc.
2. Meat, horse	125.0 g.
3. HCl (5.0%)	2.0 cc.
4. Na_2CO_3 (siccum)	3.7 g.
5. Pancreatin (Merck's glycerol)	15.0 cc.
6. NaCl	6.0 g.
7. Glycerol	
8. Agar	

Preparation:

(1) Grind up 125.0 g. of lean horse meat with 3.0 g. pepsin and 400.0 cc. distilled water with 2.0 cc. of HCl.

(2) Place in an Erlenmeyer flask and incubate at 37°C. until there is complete solution.

(3) Neutralize with Na_2CO_3 (anhydrous) and divide into 3 portions.

(4) Sterilize each portion (exact method not specified).

(5) Add to each portion with a sterile pipette 5.0 cc. of Merck's glycerol pancreatin.

(6) Keep one portion in the incubator 6 hours, another 24 hours and the other 48 hours.

(7) Sterilize in streaming steam.

(8) Neutralize with HCl.

(9) Dilute each portion to 650.0 cc. and add 2.0 g. NaCl to each portion.

(10) Add glycerol and agar as usual (amount not given).

Sterilization: Not specified.

Use: Cultivation of parasitic and saprophytic forms. Authors reported that some organisms developed better on the medium with the longer pancreatin digestion, others required a shorter period.

Variants:

(a) Bosse cultivated diphtheria bacilli on a medium prepared as follows:

(1) Free horse meat from all fat and tendons.
(2) Chop into small pieces with a knife, a scraper or meat grinding machine until there are only very loose particles.
(3) To 125.0 g. of (2) add 3.0 g. of fresh Witte-Rostock pepsin 400.0 cc. distilled water and 2.0 cc. of a 50.0% HCl solution.
(4) Place in an Erlenmeyer flask and incubate at 37°C.
(5) Incubate for 2 days, shaking occasionally and adjusting the reaction with the addition of a 50.0% HCl solution.
(6) Filter the grey white precipitate which has formed at the end of this time.
(7) Test a portion of the filtrate by the Biuret reaction (violet coloration with KOH and $CuSO_4$) which must be positive.
(8) Add 3.9 g. of Na_2CO_3 (siccum) to the filtrate.
(9) Sterilize (Method not given).
(10) Cut a hog's pancreas into small pieces with a knife, and place in the ice box for 24 hours.
(11) Add 40.0 cc., of glycerol and 160.0 cc. distilled water, and extract for several days in the ice box.
(12) Press out the juice and add a small piece of camphor to the liquid.
(13) Add 15.0 cc. of (12) to (9) by means of a sterile pipette.
(14) Incubate at 37°C. for six hours.
(15) Sterilize immediately in the autoclave, and neutralize with 50.0% HCl.
(16) Add 1950.0 cc. of water, 6.0 g. NaCl, 39.0 agar to (15).
(17) Boil in the autoclave for 3 hours.
(18) Filter thru cotton and distribute into little flasks.
(19) Sterilize as usual (exact method not given).

(b) Bosse (Kolle and Wasserman) prepared the medium as follows:
(1) Mix 150.0 g., of finely chopped horse heart with 3.0 g. of Witte's pepsin (fresh) 2.0 cc. of 50.0% HCl and 400.0 cc. of distilled water.
(2) Digest for two days at 37°C.
(3) Filter, and if positive to the Biuret test add 3.9 g. dry Na_2CO_3.
(4) Add 15.0 cc. of a glycerol extract of a hog's pancreas to (3).
(5) Incubate for 6 hours at 37°C.
(6) Sterilize in the steamer.
(7) Neutralize with HCl.
(8) Add 1950.0 cc. water, 6.0 g. NaCl and 39.0 g. agar to (7) and boil for 3 hours in the steamer.
(9) Filter thru cotton.
(10) Distribute in flasks.
(11) Sterilize.

(c) Harvey gave the following method of preparation:
(1) Mix 150.0 g. of finely minced horse heart, 5.0 g. pepsin, 2.0 cc. of 50.0% HCl and 400.0 cc. of distilled water.
(2) Leave to digest 2 days at 37°C.
(3) Filter.
(4) Add 40.0 cc. of glycerol and 160.0 cc. distilled water to a finely chopped pig pancreas.
(5) Infuse (4) for 3 days in an ice chest, adding a small piece of camphor.
(6) Add 3.9 g. anhydrous Na_2CO_3 and 15.0 cc. of (5) to the filtrate from (3).
(7) Leave 6 hours at 37°C.
(8) Sterilize at 100°C.
(9) Neutralize with HCl.
(10) Add 1950.0 cc. water, 6.0 g. sodium chloride, and 39.0 g. agar.
(11) Steam 3 hours.
(12) Filter.
(13) Distribute into test tubes and flasks.
(14) Sterilize.

(d) Deycke (Klimmer) gave the following method of preparation:
(1) Mix 125.0 g. of finely chopped horse meat with 400.0 cc. distilled water and 3.0 g. of Witte's pepsin.
(2) Add 2.0 cc. of 50.0% concentrated HCl.
(3) Incubate at 37°C. for 48 hours, adding more HCl if necessary.
(4) Filter.
(5) Add 3.9 g. water free soda to the filtrate.
(6) Sterilize (method not given).
(7) Chop the pancreas of a hog into

small pieces, and store in the ice box for 24 hours.
(8) Add 40.0 cc. of glycerol and 100.0 cc. water to (7) and place in the ice box for several days.
(9) Filter the juice from (8).
(10) Add 15.0 cc. of (9) to (6) and incubate at 37°C. for 6 hours.
(11) Sterilize (method not given).
(12) Neutralize with 5.0% HCl.
(13) Add 1950.0 cc. water, 6.0 g. NaCl, and 39.0 g. agar, and prepare as ordinary nutrient agar.

References: Deycke and Voigtländer (1901 p. 624), Bosse (1903 p. 472), Kolle and Wassermann (1912 p. 413), Harvey (1921–22, p. 100), Klimmer (1923 p. 221).

2075. Kligler's Yeast Autolysate Agar

Constituents:

1. Yeast autolysate..........	1000.0 cc.
2. Agar......................	15.0 g.

Preparation:
(1) Prepare yeast autolysate as in medium 982, but do not filter.
(2) Add agar to (1) and stir thoroly to soften agar thread or powder.
(3) Heat over a free flame to dissolve agar.
(4) Readjust the reaction to pH = 7.4.
(5) Heat on a water bath or in an Arnold for ½ hour and decant the partially clear fluid to an Erlenmeyer flask or other vessel.
(6) Cool the agar (exact temperature not given), and add one whole egg to clear.
(7) Steam in the Arnold for 30 to 45 minutes.
(8) Filter and tube.

Sterilization: Method not given.

Use: General culture medium.

Variants:
(a) Abt and Blanc solidified medium 984 with the addition of agar.
(b) Harvey prepared a similar medium as follows:
  (1) Keep 100.0 g. yeast at 50°C. for 24 hours or until liquefaction is complete by autolysis.
  (2) Dilute with water to 400.0 cc.
  (3) Steam 30 minutes.
  (4) Adjust reaction.
  (5) Filter.
  (6) Make up the volume to 1500.0 cc.
  (7) Solidify with agar.

References: Kligler (1919 p. 186), Abt and Blanc (1921 p. 452), Harvey (1921–22 p. 120).

2076. van Steenberge's Yeast Extract Agar

Constituents:

1. Yeast water agar..........	1000.0 cc.
2. Esculin....................	1.0 g.
3. Iron citrate................	1.0 g.

Preparation:
(1) Dissolve 2 and 3 in yeast water agar.

Sterilization: Not specified.

Use: Cultivation of lactic acid bacteria.

Variants: The author added 50.0 g. glucose.

Reference: van Steenberge (1920 p. 841).

2077. Jötten's Yeast Autolysate Agar

Same as medium 983, and variant (b) medium 983, but solidified by the addition of 2.2% agar.

2078. Couret and Walker's Autolyzed Tissue Agar

Constituents:
1. Agar solution.
2. Tissue.

Preparation:
(1) Collect bits of sterile liver, kidney and brain of healthy rabbits, guinea pigs, kittens and human placenta, in sterile flasks.
(2) Seal and place in the thermostat at 40°C. for 10 to 20 days.
(3) Titrate the fluid tissue to a neutral or slightly acid reaction (not exceeding 1.5% acid) by the addition of N/1 NaOH.
(4) The agar is to be prepared in the usual manner (exact method not given) but is to contain no peptone, beef extract, etc.
(5) Adjust the reaction to neutral or to an alkalinity not to exceed 0.5%. Tube and slant.
(6) Distribute 0.1 to 0.5 cc. of (3) (autolyzed tissue) over the surface of each agar slant.
(7) Keep several hours in the horizontal position to allow the agar to absorb the tissue juice.
(8) Incubate to determine sterility.

(9) The agar may be melted, and cooled to 50°C. and the tissue juice added. In this case it is necessary to break the surface of the slants before using.

Sterilization: Not specified.

Use: Cultivation of amoeba.

References: Couret and Walker (1913 p. 253), Stitt (1923 p. 51).

SUBGROUP II-C. SECTION 8

Basal or complete media containing agar with constituents of plant origin (exclusive of digests) of unknown chemical composition. (Animal products may also be present.)

A_1. Containing bacteria, yeast, other fungi or their derivatives.

Gassner's Yeast Autolysate Agar... 2079
Harvey's Yeast Extract Agar...... 2080
Beijerinck's Yeast Water Agar..... 2081
Duval's Parasite Agar.............. 2082
Gassner's Asparagin Yeast Water Agar.............................. 2083
v. Eisler's Bacterial Emulsion Agar. 2084
Lanken and Meyers' Fungus Infusion Agar........................ 2085
Ayers and Mudge's Milk Powder Yeast Agar..................... 2086

A_2. Containing flowering plants or their derivatives.

B_1. Non leguminous plants used.

C_1. Grains or their derivatives used.

D_1. Malts, beerworts or their derivatives added.

Park, Williams and Krumwiede's Basal Beer Wort Agar............ 2087
Heinemann's Beer Wort Agar...... 2088
Groenewege's Indican Malt Agar... 2089
Peklo's Malt Infusion Agar........ 2090
Fulmer and Grimes' Malt Infusion Agar.............................. 2091
Rettger, Reddish and McAlpine's Malt Extract Agar............... 2092
Tanner and Deck's Acid Near Beer Agar.............................. 2093
Janke's Lager Beer Agar........... 2094
Sobel's Lactose Beer Agar.......... 2095
Difco Wort Agar (Dehydrated)..... 2096

D_2. Materials other than D_1.

Meacham, Hopfield and Acree's Corn Meal Agar....................... 2097
Buchanan's Silage Agar............ 2098
Reed and Colley's Corn Meal Agar... 2099
Barlow's Sucrose Corn Agar (Tanner)......................... 2100
Pinoy's Flax Seed Agar............ 2101
Omeliansky and Ssewerowa's Flax Fiber Agar....................... 2102
Heider's Wheat Agar (Klimmer).... 2103
Klimmer's Oat Agar................ 2104
Bacto Corn Meal Agar (Dehydrated). 2105

C_2.* Fruits or their derivatives used.

Milburn's Basal Prune Agar........ 2106
Reed and Cooley's Prune Agar..... 2107
Bacto Prune Agar (Dehydrated)... 2108
Dombrowski's Raisin Must Agar... 2109
Perold's Grape Juice Agar.......... 2110
Richter's Meat Infusion Wine Agar.. 2111
Jenkins' Tomato Infusion Agar..... 2112

C_3. Tubers or their derivatives used.

D_1. Not containing animal products.

Graham-Smith's Potato Agar....... 2113
Dawson's Potato Juice Agar........ 2114
Gaehtgens' Potato Agar............. 2115
Bacto Potato Dextrose Agar (Dehydrated)......................... 2116
Kellerman and McBeth's Potato Agar.............................. 2117
Lubinski's Glycerol Potato Agar... 2118
Ficker's Glycerol Potato Juice Agar.............................. 2119
Weinzirl's Sucrose Potato Water Agar.............................. 2120
Rochaix's Carrot Agar............. 2121
Schardinger's Hay Infusion Agar.... 2122

D_2. Containing animal products.

Bordet and Gengou's Potato Blood Agar (Roos)..................... 2123

C_4. Non leguminous plants or their derivatives other than C_1 to C_3 used.

Schardinger's Straw Infusion Agar. 2124
Reed and Cooley's Spinach Agar... 2125
Owen's Cane Juice Agar............ 2126
Owen's Molasses Agar.............. 2127
Le Fevre and Round's Cucumber Juice Agar....................... 2128

B_2. Leguminous plants or derivatives employed.

C_1. Seeds used.

Bacto Lima Bean Agar (Dehydrated)......................... 2129
Stutzer's Basal Legume Seed (Infusion) Agar..................... 2130
Wyant and Tweed's Pea Agar...... 2131
Kaufmann's Jequirity Seed Agar... 2132
Mazé's Sucrose Bean Agar......... 2133
Reed and Cooley's Lima Bean Agar.. 2134

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* See C_3 and C_4.

Thom's Bean Agar (Tanner)....... 2135
Stutzer's Bean Infusion Agar....... 2136
Vogel and Zipfel's Legume Flour Agar (Klimmer).................. 2137
C_2. Materials other than seeds used.
Bacto Bean Pod Agar (Dehydrated). 2138
Didlake's Soy Bean Root Agar..... 2139
Zipfel's Legume Leaf Agar.......... 2140
Simon's Legume Agar (Klimmer and Krüger)......................... 2141

2079. Gassner's Yeast Autolysate Agar

Constituents:

1. Water.
2. Yeast, Brewer's.
3. Agar (3.0%).
4. NaCl.

Preparation:

(1) Place about 10 liters of brewer's yeast in a flask and wash with water. Allow to stand for 30 minutes and pour off the liquid.

(2) Repeat the washing process until the wash water is no longer brown, but slightly turbid. Washing five times is usually sufficient.

(3) After pouring off the last water add 18 liters of water to the remaining washed yeast cells.

(4) Boil in the autoclave or steamer as in the preparation of meat bouillon.

(5) Allow to stand for a suitable length of time and remove the liquid from the sediment or filter thru filter paper.

(6) This bouillon may be used exactly in the same manner as meat bouillon.

(7) To prepare agar dissolve 30.0 g. of agar and 5.0 g. NaCl in 1000.0 cc. of (6).

(8) Adjust to slightly alkaline to litmus.

Sterilization: Not specified.

Use: General inexpensive culture medium.

Variants: The author added 0.3 or 0.5% lactose.

Reference: Gassner (1916–17 p. 311).

2080. Harvey's Yeast Extract Agar

Constituents:

1. Water.....................	1000.0 cc.
2. Yeast (bakers or brewers)..	100.0 g.
3. Agar (2.5% solution)	

Preparation:

(1) Mix one part bakers or brewers yeast with ten parts water.

(2) Heat the mixture 20 minutes at a temperature not exceeding 50°C.

(3) Steam 2 hours.

(4) Filter and refilter thru well-wetted, thick, filter paper.

(5) Mix two parts (4), cooled to 15°C. with three parts melted 2.5% agar solution of a reaction pH = 7.4. (Agar may have peptone and NaCl in it if desired.)

Sterilization: Sterilize in the autoclave.

Use: Cultivation of meningococci. Harvey reported that with a semi-liquid agar (0.5%) viability can be preserved in stab cultures much longer than with a stiffer agar.

Reference: Harvey (1921–22 p. 120).

2081. Beijerinck's Yeast Water Agar

Constituents:

1. Water tap...........	1000.0 cc.
2. Yeast................	80.0 g.
3. Agar.................	0.75 g.
4. Glucose..............	5.0 to 10.0 g.
5. Chalk	

Preparation:

(1) Boil 8.0 yeast in 100.0 cc. tap water.

(2) Dissolve 0.75 g. agar and 5.0 to 10.0 g. glucose in (1) by boiling.

(3) Filter.

(4) Add a layer of pure chalk suspension.

Sterilization: Not specified.

Use: To study the fermentation of glucose by bacteria.

Reference: Beijerinck (1891 p. 782).

2082. Duval's Parasite Agar

Constituents:

1. Water.....................	1000.0 cc.
2. Agar	
3. *Entameba coli*	
4. *B. typhosus*	

Preparation:

(1) Prepare a solution of agar (amount not given) in water.

(2) Adjust to 1.0% alkaline to phenolphthalein.

(3) Tube.

(4) Incubate the sterile slants of (3) with a mixture of *Entameba coli* and *B. typhosus*.

(5) Incubate at room temperature for 48 hours.

Sterilization: Method of sterilization of (3) not given.

Use: Cultivation of *B. leprae*. Inoculate the slant with an emulsion prepared from leprous tissue. The author reported that the organism grew without the presence of *B. typhosus*.

Reference: Duval (1910 p. 653).

2083. Gassner's Asparagin Yeast Water Agar

Constituents:

1. Water	900.0 cc.
2. Yeast water	900.0 cc.
3. Agar	30.0 g.
4. NaCl	5.0 g.
5. Asparagin	10.0 g.
6. Water blue (0.5%)	250.0 cc.

Preparation:

(1) Prepare yeast water.
(2) Mix equal parts yeast water and water.
(3) Dissolve 30.0 g. agar, 10.0 g. asparagin, and 5.0 g. NaCl in (2).
(4) To each 80.0 cc. of (3) add 20.0 cc. of a 0.5% solution of water blue.

Sterilization: Not specified.

Use: To study the nitrogen requirements for growth of the colon-typhoid and dysentery group. The author reported that asparagin was utilized as a nitrogen source by the typhoid and dysentery group as well as by *Bact. coli*.

Variants: The author added 0.1, 0.5 or 1.0% glucose.

Reference: Gassner (1917–18 p. 260).

2084. v. Eisler's Bacterial Emulsion Agar

Constituents:

1. Water	100.0 cc.
2. NaCl (0.5%)	0.5 g.
3. Agar	3.0 g.
4. Bacterial emulsion	

Preparation:

(1) Dissolve 3.0% agar in a 0.5% NaCl solution.
(2) The reaction is usually slightly alkaline.
(3) Distribute in 5.0 cc. lots and sterilize (method not given).
(4) Prepare bacterial emulsions by washing bacteria (*B. coli*, *typhosus*, and *Staphylococcus pyogenes aureus* were used) from large 24 hour agar surfaces with 25 to 30.0 cc. salt solution.
(5) Heat the emulsion for an hour and a half at 56°C. or boil for one-half hour.
(6) Add from 1.0 to 3.0 cc. of the emulsion to each sterile tube of agar melted and cooled to 50°C.

Sterilization: Method of sterilization of agar not given. See step (5) for sterilization of bacterial emulsion.

Use: To study growth of bacteria on killed bacteria. Author reported that *B. coli* grew only fairly well. Did not grow at all on the sediment. Grew well on staphylococcus organisms, but poorly on the sediment. Scant growth of *B. coli* on *B. typhosus* cells. *Staphylococcus aureus* grew well on *B. coli*, *typhosus* or *Staphylococcus* emulsion; *B. typhosus* grew on *Staphylococcus aureus* emulsion, but very poorly on *B. coli* or *typhosus* emulsions.

Variants: The author specified that the emulsion might be centrifuged for quite a long time and the supernatant, cell free opalescent fluid be added to the agar, or the sediment be mixed with a volume of NaCl solution, equal to that removed from the sediment, and added to the agar.

Reference: v. Eisler (1918 p. 197).

2085. Lanken and Meyers' Fungus Infusion Agar

Solidify medium 1150 and variant (b) medium 1150 by the addition of 2.0% agar.

2086. Ayers and Mudge's Milk Powder Yeast Agar

Constituents:

1. Water	250.0 cc.
2. Skimmed milk powder	5.0 g.
3. Na_2HPO_4–$2H_2O$ (Sorensen's phosphate)	1.0 g.
4. Yeast (dry fresh)	10.0 g.
5. Agar (3.0% washed)	500.0 cc.

Preparation:

(1) Pour 5.0 g. skimmed milk powder into 20.0 cc. cold distilled water. Stir until solution is complete.
(2) Dissolve 1.0 g. Sorensen's phosphate ($Na_2HPO_4.2H_2O$) in 5.0 cc. distilled water by heating.
(3) Add (2) to (1), place at 30°C. in a water bath and heat to 60°C. in 10 minutes. A flocculent grayish precipitate appears.

(4) Steam until the precipitate turns white.
(5) Dilute about one-third with distilled water and steam 5 more minutes.
(6) Decant the solution and filter, keeping most of the precipitate in the beaker until most of the liquid has passed thru the filter.
(7) Wash the precipitate with a little distilled water. The filtrate is cloudy.
(8) Make up to 250.0 cc. by the addition of distilled water.
(9) Steam 10.0 g. yeast in 100.0 cc. distilled water for 45 minutes in an Arnold. Filter until filtrate is clear and brilliant.
(10) Wash with 100.0 cc. distilled water.
(11) Make up to 250.0 cc. with distilled water.
(12) Place 30.0 g. agar in 2000.0 cc. distilled water and let stand for 24 hours.
(13) Pour off the water and add an equal volume of fresh water.
(14) Allow to stand for 24 hours, pour on cotton flannel cloth in a funnel and wash with a liter of distilled water. Press out as much water as possible.
(15) Add enough water to have the total weight 1030.0 g.
(16) Dissolve the agar by heating in the Arnold, and filter thru cotton flannel until clear.
(17) Mix equal parts of (16) and (8).
(18) The usual pH = 6.7.

Sterilization: Method not specified.

Use: To determine the bacterial count of milk.

Variants: Any desired indicator may be added.

Reference: Ayers and Mudge (1920 p. 581).

2087. Park, Williams and Krumwiede's Basic Beer Wort Agar

Constituents:

1. Beer Wort	1000.0 cc.
2. Agar (1.5%)	15.0 g.

Preparation:

(1) Dissolve 2.0% of any of the added nutrients in beer wort (see medium 1218) that has been solidified by the addition of 1.5% agar.

Sterilization: Not specified.

Use: Cultivation of molds.

Added nutrients: The authors added 2.0% of any desired carbohydrate, alcohol, etc.

Reference: Park, Williams and Krumwiede (1924 p. 134).

2088. Heinemann's Beer Wort Agar

Constituents:

1. Beer wort	1000.0 cc.
2. Agar 1.5%	15.0 g.

Preparation:

(1) Autoclave beer wort at 120° for 5 minutes.
(2) Cool.
(3) Filter.
(4) Dissolve 1.5% agar in the filtrate.
(5) Clarify with the white of egg.

Sterilization: Not specified.

Use: Cultivation of molds, yeast, etc.

Variants:

(a) Dombrowski cultivated milk yeast in a medium prepared by dissolving 1.5% agar in wort.
(b) Janke cultivated acetic acid bacteria found in beers on a medium prepared by dissolving 2.0% agar and 2.0% gelatin in hopped Lager beer wort of 12.75° Ballings, clarified with egg white, filtered thru cotton, distributed in 50.0 cc. lots and sterilized by the discontinuous method in the steamer.
(c) Harvey cultivated trichophyta, molds, yeasts, etc., in a medium prepared by dissolving 1.5% agar in beer wort diluted to a specific gravity of 1.1.

References: Heinemann (1905 p. 128), Dombrowski (1910 p. 380), Löhnis (1913 p. 88), Janke (1916 p. 6), Harvey (1921–22 p. 113), Klimmer (1923 p. 207), Park, Williams and Krumwiede (1924 p. 134)

2089. Groenewege's Indican Malt Agar

Constituents:

1. Malt agar	100.0 cc.
2. Indican (0.1%)	1.0 g.

Preparation:

(1) Prepare malt agar.
(2) Add 0.1% indican to (1).

Sterilization: Not specified.

Use: Cultivation of *Phytobacter lycopersicum* n. sp. (causing tomato rot). Author reported that the indican was decomposed.

Reference: Groenewege (1913 p. 24).

2090. Peklo's Malt Infusion Agar

Same as medium 1214 but solidified by the addition of 1.0% agar.

2091. Fulmer and Grimes' Malt Infusion Agar

Constituents:

1. Distilled water	1150.0 cc.
2. Malt, distillers	360.0 g.
3. Agar (1.5%)	15.0 g.

Preparation:

(1) Mash at 55°C. 360.0 g. malt with 1150.0 cc. distilled water for 24 hours.
(2) Filter thru towelling, then thru filter paper.
(3) Heat for 30 minutes under 15 pounds pressure.
(4) Allow to stand 3 days and filter.
(5) Add agar.
(6) Heat in autoclave 30 minutes at 15 pounds.
(7) Filter thru absorbent cotton and tube.

Sterilization: Sterilize in live steam for 30 minutes on 2 successive days.

Use: Growth of yeast *Sacch. cerevisiae* and *Torula sphaerica*.

Reference: Fulmer and Grimes (1923 p. 586).

2092. Rettger, Reddish and McAlpine's Malt Extract Agar

Constituents:

1. Water	900.0 cc.
2. Malt extract powder, Difco	100.0 g.

Preparation:

(1) Dissolve 2 in 1.
(2) Reaction is about pH = 5.5 to 5.6.

Sterilization: Not specified.

Use: Isolation of yeast from feces. Authors reported that the reaction was too acid for the development of bacteria.

Reference: Rettger, Reddish and McAlpine (1924 p. 328).

2093. Tanner and Deck's Acid Near Beer Agar

Constituents:

1. Distilled water	600.0 cc.
2. Malt drink (near beer)	400.0 cc.
3. Agar	15.0 g.
4. Lactic acid (5.0% soln.)	

Preparation:

(1) Add 15.0 g. agar to 400.0 cc. malt drink (near beer) and 600.0 cc. distilled water.
(2) Boil to dissolve the agar.
(3) Tube.
(4) Just previous to use add 0.4 cc. of 5.0% sterile solution of lactic acid to each sterile tube of (3).

Sterilization: Method of sterilization of agar or lactic acid not given.

Use: Cultivation of yeast causing sore throat probably *Endomyces* or *Monilia*.

Reference: Tanner and Deck (1923–24 p. 285).

2094. Janke's Lager Beer Agar

Constituents:

1. Lager beer.
2. Gelatin.
3. Alcohol.
4. Agar.

Preparation:

(1) Evaporate lager beer to one-half its original volume by steaming.
(2) Add water to its original volume.
(3) Add 2.0% agar and 2.0% gelatin to (2).
(4) Clarify with white of egg.
(5) Filter thru cotton.
(6) Make up to the original volume by the addition of water.
(7) Distribute in 10.0 cc. lots in Freudenreich flasks.

Sterilization: Sterilize on each of 3 successive days in the steamer.

Use: Cultivation of acetic acid bacteria from beers.

Reference: Janke (1916 p. 6).

2095. Sobel's Lactose Beer Agar

Constituents:

1. Water	500.0 cc.
2. Beer	500.0 cc.
3. Agar	15.0 g.
4. Lactose	5.0 g.
5. Congo red	1.0 g.

Preparation:

(1) Boil 500.0 cc. of beer, (preferably stale Pilsner beer) for 5 minutes.
(2) Cool and bring to 1 liter volume by the addition of water.
(3) Add 15.0 g. agar and allow to soak for

2 hours. Then boil until the agar has dissolved.
(4) Add 5.0 g. of dry sterilized lactose to boiling (3) and 1.0 g. of dry sterilized congo red.
(5) Boil vigorously for several minutes and add the amount of water lost by evaporation.
(6) Pour directly into sterile Petri dishes if the medium is to be used at once.

Sterilization: If the medium is not to be used at once sterilize in the steamer on two successive days.

Use: Diagnosis of cholera, typhoid fever and dysentery. The author gave the following reactions:

ORGANISM	CONGO RED	LITMUS
Cholera	Red-luxuriant growth	Dark blue
Typhoid	Light pink	Light blue
Dysenteriae (Flexner)	Blue violet	Blue
Coli	Reddish brown or black	Red

Variants: The author used 1.0 g. of litmus puriss instead of congo red.

Reference: Sobel (1915 p. 1573).

2096. Difco Wort Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Maltose, Technical, Difco	12.75 g.
3. Malt extract, Difco	15.0 g.
4. Dextrin, Difco	2.75 g.
5. Glycerol, C. P	2.35 g.
6. K_2HPO_4	1.0 g.
7. NH_4Cl	1.0 g.
8. Peptone, Bacto	0.78 g.
9. Agar, Bacto	15.0 g.

Preparation:
(1) Dissolve 50.63 g. Difco Wort Agar (Dehydrated) in 1000.0 cc. distilled water by boiling or autoclaving.
(2) Distribute as desired.
(3) If sterilized at 15 pounds pressure for 20 minutes pH = 4.7±.

Sterilization: Sterilize at 15 pounds for 20 minutes.

Use: Cultivation of yeasts.

Reference: Digestive Ferments Co. (1925 p. 14).

2097. Meacham, Hopfield and Acree's Corn Meal Agar

Constituents:

1. Water	1000.0 cc.
2. Corn meal, yellow	7.5 g.
3. Agar	25.0 g.
4. K_2HPO_4 to M/50	
5. Acetic acid to M/50	

Preparation:
(1) Cook 7.5 g. yellow corn meal with 200.0 cc. water in water bath at 60° C. for one hour.
(2) Allow meal to settle, decant the liquid and mix fresh water with the meal.
(3) Decant this liquid and mix the two extracts.
(4) Dissolve 3, 4 and 5 in (3) and make up to 1000.0 cc.
(5) Reaction of about pH = 5.7.

Sterilization: Not specified.

Use: Study phosphate-acetate and phosphate-phthalate as buffers. Authors reported that potassium acid phthalate showed best growth of *E. parasitica.*

Variants: The authors used M/50 potassium acid phthalate instead of M/50 acetic acid.

Reference: Meacham, Hopfield and Acree (1920 p. 307).

2098. Buchanan's Silage Agar

Constituents:

1. Water, tap	1000.0 cc.
2. Silage	500.0 g.
3. Agar (1.5%)	15.0 g.

Preparation:
(1) Boil 500.0 g. fresh silage for 30 minutes with a liter of tap water.
(2) Filter. Wash the silage on the filter with hot water until a liter of the decoction is secured.
(3) Autoclave with 1.5% agar until solution is complete.
(4) Filter.
(5) Tube.

Sterilization: Method not given.

Use: Cultivation of *Monascus purpurens* from silage.

Reference: Buchanan (1910 p. 100).

2099. Reed and Cooley's Corn Meal Agar

Constituents:

1. Water	500.0 cc.

2.	Corn meal	50.0 g.
3.	Agar (1.5 or 2.0%)	7.5 to 10.0 g.

Preparation:

(1) Allow 50.0 g. of corn meal to simmer in 500.0 cc. of water for two hours. (*Phaseolus lunatus*, lime bean meal may be used instead of corn meal.)
(2) Strain thru a cloth.
(3) Add 1.5 to 2.0% agar.
(4) Melt and filter thru cotton.

Sterilization: Sterilize fractionally on three successive days.

Use: Cultivation of *Heterosporium variabile.* Authors reported this a very good medium for fungi.

Reference: Reed and Cooley (1911–12 p. 49).

2100. Barlow's Sucrose Corn Agar (Tanner)

Constituents:

1.	Water	4000.0 cc.
2.	Corn	1000.0 g.
3.	Sucrose	80.0 g.
4.	Agar	60.0 g.
5.	Litmus	100.0 g.

Preparation:

(1) Stir 1000.0 g. of corn into boiling water.
(2) Add 3, 4 and 5 to (1).
(3) Boil 5 minutes.
(4) Heat in an autoclave and cool slowly. The heavy matter settles off.
(5) Draw off and tube the supernatant agar.

Sterilization: Not specified.

Use: General culture medium.

Reference: Tanner (1919 p. 50).

2101. Pinoy's Flax Seed Agar

Constituents:

1.	Water	1000.0 cc.
2.	Agar	20.0 g.
3.	Flax seed	50.0 g.

Preparation:

(1) Prepare a medium from 1 liter of water, 20.0 g. of agar and 50.0 g. flax seed. (Method not given.)
(2) Heat at 117°C.
(3) Distribute in culture apparatus.

Sterilization: Sterilize at 115°C. for 15 minutes.

Use: Cultivation of *Bacterium fimbriatum.*

Reference: Pinoy (1907 p. 626).

2102. Omeliansky and Ssewerowa's Flax Fiber Agar

Constituents:

1.	Water	1000.0 cc.
2.	Flax fiber	50.0 g.
3.	Mannitol	20.0 g.
4.	Potassium phosphate	0.2 g.
5.	Agar	15.0 g.

Preparation:

(1) Heat 50.0 g. of flax at 115°C. in the autoclave with a liter of water.
(2) Filter.
(3) Neutralize to slightly alkaline reaction by adding Na_2CO_3.
(4) Dissolve 3, 4 and 5 in (3).

Sterilization: Not specified.

Use: To study pigment production of azotobacter, *Azotobacter chroococcum.* Authors reported that on the medium given above brown pigment appeared after ten days. The substitution of flax extract for water increased the luxuriance of growth of Azotobacter, Clostridium, Pasteurianum, *Bac. cellulose*, Nitrobacter and other forms.

Variants: The authors reported that using a medium prepared as above but substituting 20.0 to 30.0 g. of dextrin for mannitol, and adding 20.0 g. of chalk that pigment was produced after 3 to 4 days. The addition of the chalk favored the production of pigment. Pigment more luxuriant in the variant than in the medium given above.

Reference: Omeliansky and Ssewerowa (1911 p. 645).

2103. Heider's Wheat Agar (Klimmer)

Constituents:

1.	Water	1000.0 cc.
2.	Wheat	500.0 g.
3.	Agar (1.5%)	15.0 g.

Preparation:

(1) Soak 500.0 g. of wheat in a liter of water for 24 hours.
(2) Filter.
(3) Dissolve 1.5% agar in the filtrate.
(4) Neutralize to litmus.

Sterilization: Not specified.

Use: Spore production by Anthrax bacilli.

Variants: Klimmer prepared a similar medium as follows:

(1) Macerate 500.0 g. of wheat with a liter of water for 24 hours.

(2) Filter.
(3) Make up the filtrate to a liter.
(4) Dissolve 5.0 g. NaCl and 17.5 g. of agar in (3).
(5) Make slightly alkaline to phenolphthalein.
(6) Sterilization not specified.

Reference: Klimmer (1923 p. 207).

2104. Klimmer's Oat Agar

Same as the variant of medium 2103, but using oats instead of wheat.

2105. Bacto Corn Meal Agar (Dehydrated)

Constituents:
1. Distilled water.............
2. Corn Meal Agar (Bacto).... 20.0 g.

Preparation:
(1) Dissolve 20.0 g. Bacto corn meal agar (Dehydrated) in 1000.0 cc. distilled water by boiling or autoclaving, preferably the latter. Bacto corn meal agar is a synthetic medium representing the water soluble ingredients of corn meal solidified by the addition of 1.5% agar.
(2) Distribute as desired.

Sterilization: Sterilize for 20 minutes at 15 pounds pressure.

Use: General culture medium.

Reference: Digestive Ferments Co. (1925 p. 17).

2106. Milburn's Basal Prune Agar

Constituents:
1. Water...................... 1000.0 cc.
2. Agar
3. Prunes..................... 1 lb.

Preparation:
(1) Soak 1.0 pound of dry prunes in 1 liter of water for 12 hours.
(2) Filter thru a towel and then cotton.
(3) Concentrate to a specific weight of 1.06 to 1.08.
(4) Prepare media using 40.0% of the prune infusion, one of the added nutrients and solidify with agar (method not given).

Sterilization: Not specified.

Use: To study color formation.

Added nutrients: The author added one of the following materials and gave the following reactions:
(a) glucose 1.0, 2.0 or 5.0%.
(b) tartaric acid 0.2 or 0.5%.
The glucose seemed to suppress the conidia development. There was no yellow color with either glucose or tartaric acid. Color was dark green using tartaric acid.
(c) NH_4NO_3 0.5 or 1.0%.
(d) KNO_3 0.5 or 1.0%.
(e) K_3PO_4 0.5 or 1.0%.
(f) K_2HPO_4 0.5 or 1.0%.
(g) KH_2PO_4 0.5 or 1.0%.
(h) Dipotassium ammonium phosphate.
(i) tyrosine.
(j) leucine.
(k) oxamide.
(l) legrimin.
(m) casein.
(n) peptone.
(p) blood serum.
(q) meat extract.
(r) asparagin.
(s) acetamide.
(t) creatin.
(u) albumin.
(v) glucose.
(w) nuclean.
Good growth and conidia were colored dirty green and greyish white with tyrosine, asparagin, leucine and acetamide. Little growth and no color with oxamide. Creatin, legumin albumin, casein and blood serum gave good growth a greenish and yellow color. Remaining materials gave excellent growth of a yellow color.
(x) glucose 5.0 to 40.0%.
High concentrations of glucose tended to slow up growth and no color was formed.
(y) Knops' nutrient solution 0.25, 0.5, 1.0, 2.0 or 5.0%.
(z) Knops' nutrient solution 0.25, 0.5, 1.0, 2.0 or 5.0% + glucose (amount of glucose not given).
The presence of Knops' mineral salts seemed to aid the conidia production. Hence more greenish color was produced. More green was produced without dextrose than with it present.

Reference: Milburn (1904 p. 133).

2107. Reed and Cooley's Prune Agar

Constituents:
1. Water...................... 600.0 cc.

2. Prunes	100.0 g.
3. Agar (1.5%)	9.0 g.

Preparation:
(1) Boil 100.0 g. of dried prunes in 600.0 cc. water.
(2) Filter.
(3) Dissolve 1.5% agar in (2).

Sterilization: Sterilize by the fractional method in the steamer.

Use: Cultivation of *Heterosporium variabile.* Author reported that the fungus grew profusely on this medium and bacterial contamination was easily prevented.

Variants: Klimmer used 100.0 g. prunes in 500.0 cc. of water.

Reference: Reed and Cooley (1911–12 p. 50), Klimmer (1923 p. 206).

2108. Bacto Prune Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Bacto prune agar	24.0 g.

Preparation:
(1) Dissolve 24.0 g. of Bacto prune agar in 1000.0 cc. distilled water by boiling or autoclaving, preferably the latter. Bacto prune agar represents a clarified infusion of the best grade of prunes, made solid by the addition of agar.
(2) Distribute as desired.

Sterilization: Sterilize for 20 minutes at 15 pounds pressure.

Use: General culture medium.

Reference: Digestive Ferments Co. (1925 p. 17).

2109. Dombrowski's Raisin Must Agar

Constituents:

1. Raisin must	1000.0 cc.
2. Agar (1.5%)	15.0 g.

Preparation:
(1) Dissolve 1.5% agar in raisin must.

Sterilization: Not specified.

Use: Cultivation of milk yeast.

Reference: Dombrowski (1910 p. 380).

2110. Perold's Grape Juice Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	15.0 g.
3. Gelatin	10.0 g.
4. Grape juice	60.0 cc.

Preparation:
(1) Dissolve 15.0 g. agar in 1000.0 cc. distilled water.
(2) Add 10.0 g. of gelatin to (1).
(3) Filter thru a towel in the steamer (requires about an hour).
(4) To each 100.0 cc. of (3) add 6.0 cc. of grape juice.

Sterilization: Sterilize for one hour in the steamer.

Use: Cultivation of bacteria from wine.

Reference: Perold (1909 p. 18).

2111. Richter's Meat Infusion Wine Agar

Constituents:

1. Water	250.0 cc.
2. Meat	250.0 g.
3. Agar agar	10.0 g.
4. Moselle wine	150.0 cc.
5. Gelatin (2.0%)	7.0 g.

Preparation:
(1) Pour 250.0 cc. of water over finely chopped meat and allow to infuse over night. (Temperature not specified.)
(2) Add 150.0 cc. of Moselle wine to 10.0 g. of shredded agar.
(3) Allow to stand several hours and then heat to boiling in a water bath. Heat until solution is complete.
(4) Allow to stand over night (solidification takes place).
(5) On the following morning, liquefy the agar by heating.
(6) Neutralize (5) by the addition of Na_2CO_3 solution.
(7) Dissolve 2.0% gelatin in the infusion from (1).
(8) Mix liquid (6) and (7) and boil for 15 minutes.
(9) Filter thru a simple filter using a hot water funnel. (It may be necessary to refilter the first 20.0 or 30.0 cc.)
(10) Refilter thru the same filter until all turbidity has disappeared.

Sterilization: Not specified.

Use: Culture medium for anthrax, cholera, typhoid and chicken cholera bacilli. Author reported that medium is slightly yellow but clear and transparent.

Reference: Richter (1887 p. 600).

2112. Jenkins' Tomato Infusion Agar

Constituents:

1. Water.

2. Tomato.
3. Agar.

Preparation:

(1) Place small pieces of tomato in a glass jar and add sufficient water to just cover the topmost layer.
(2) Steam for 2 hours.
(3) Filter.
(4) Add 1.6% agar to the filtrate.
(5) Heat at 120°C. for 30 minutes.
(6) Add 40.0% NaOH to obtain a reaction of a minus 5 on the Eyres scale.
(7) Filter and resterilize the medium in the autoclave.
(8) Readjust the reaction to a minus 5.
(9) Tube while hot.

Sterilization: Final sterilization not specified.

Use: Cultivation of streptococci and pneumococci.

Variants: Green or canned tomatoes may be used instead of fresh red ones.

Reference: Jenkins (1923 p. 116).

2113. Graham-Smith's Potato Agar

Constituents:

1. Water	500.0 cc.
2. Potato pulp	500.0 g.
3. Agar	30.0 g.

Preparation:

(1) Wash and peel potatoes and crush in a mincing machine.
(2) Add water in the ratio of 1.0 g. of potato pulp to 1.0 cc. of water.
(3) Allow to stand in a flask for 12 hours.
(4) Filter thru a filter paper.
(5) The medium may be left in the acid condition or to give an alkaline medium, neutralize the acid with N/1 caustic soda, using litmus as the indicator and then adding 3.0 cc. of alkali per liter.
(6) Treat agar as usual (treatment not specified) and add 3.0% to (4).
(7) Place in steam sterilizer at 100°C. until agar is dissolved.
(8) When cool add white of egg and clarify in the steam sterilizer.
(9) Filter thru Chardin filter paper.
(10) Tube.

Sterilization: Sterilize on 3 successive days in steam at 100°C.

Use: Differentiation of diphtheria and diphtheria-like bacilli. Author reported that diphtheria bacilli colonies after 24 hours at 37°C. were either opaque or transparent. Pseudo-bacilli formed medium sized, round, whitish, opaque dome shaped colonies. Acid agar clear and opalescent. Alkaline agar clear but of a brownish color.

Variants: Thomas (Tanner) prepared a similar medium as follows:

(1) Wash, pare and slice potatoes.
(2) Heat one volume of (1) in two volumes of water slowly for 2 hours.
(3) Boil, after heating for two hours.
(4) Filter thru cloth.
(5) Add water to make up the loss due to evaporation.
(6) Filter.
(7) Add 1.0% shredded agar to the filtrate.
(8) Heat in the autoclave for 30 minutes at 15 pounds pressure.
(9) Filter, if desired, and tube.
(10) Sterilization not specified.

References: Graham-Smith (1904 p. 278), Tanner (1919 p. 61).

2114. Dawson's Potato Juice Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	20.0 g.
3. Potato juice 500.0 g.	

Preparation:

(1) Use 500.0 g. unskinned potatoes and 1000.0 cc. water and obtain potato juice.
(2) Free juice from starch (method not given).
(3) Dissolve agar in (2).

Sterilization: Not specified.

Use: To show bacterial variation of *B. coli*.

Reference: Dawson (1919 p. 142).

2115. Gaehtgens' Potato Agar

Constituents:

1. Water	1000.0 cc
2. Potato	500.0 g.
3. Agar	
4. NaCl	

Preparation:

(1) Carefully wash and peel 500.0 g. potatoes.
(2) Grind the potatoes fine by means of a porcelain mortar. This is to be

carried out under the water as far as possible.
(3) Allow to stand for 3 hours at room temperature.
(4) Filter thru a clean towel.
(5) Heat at 100°C. for one hour.
(6) Filter thru filter paper.
(7) Dissolve NaCl and agar (amounts not given) in (6).
(8) Adjust to the desired reaction by the addition of soda.

Sterilization: Sterilize for one hour in the autoclave (temperature or pressure not given).

Use: Inexpensive culture medium.

Variants: The potato water may be sterilized for an hour in the autoclave and kept until ready for use, and then add the NaCl and agar as above.

Reference: Gaehtgens (1916 p. 46).

2116. Bacto Potato Dextrose Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Potato infusion, dry	5.0 g.
3. Glucose, Bacto	20.0 g.
4. Agar, Bacto	15.0 g.

Preparation:
(1) Dissolve 40.0 g. of Bacto Potato Dextrose Agar (Dehydrated) in 1000.0 cc. of distilled water by boiling or autoclaving, preferably the latter. The dry potato infusion is prepared by infusing 50 parts dried potatoes.
(2) Distribute.

Sterilization: Sterilize at 15 pounds pressure for 20 minutes.

Reference: Digestive Ferments Co. (1925 p. 17).

2117. Kellerman and McBeth's Potato Agar

Constituents:

1. Water	1000.0 cc.
2. Potato	100.0 g.
3. K_2HPO_4	1.0 g.
4. $MgSO_4$	1.0 g.
5. NaCl	1.0 g.
6. $(NH_4)_2SO_4$	2.0 g.
7. $CaCO_3$	2.0 g.
8. Agar	10.0 g.

Preparation:
(1) Peel, steam and mash a quantity of potatoes.
(2) To 100.0 g. of (1) add 800.0 cc. of water and steam for 30 minutes.
(3) Filter thru cotton.
(4) Dissolve 3, 4, 5, 6 and 7 in 1000.0 cc. of water.
(5) Mix 500.0 cc. of (4) and 500.0 cc. of the filtrate from (3).
(6) Dissolve 10.0 g. of agar in (5).

Sterilization: Not specified.

Use: Cultivation of *Bacillus flavigens*, *Bacillus amylolyticus*, *Bacillus rossica*.

Variants: Tanner used 15.0 g. agar instead of 10.0 g.

References: Kellerman and McBeth (1912 p. 487), Tanner (1919 p. 60).

2118. Lubinski's Glycerol Potato Agar

Constituents:

1. Water	1500.0 cc.
2. Potato	1000.0 g.
3. Glycerol	60.0 g.
4. Agar	15.0 to 20.0 g.

Preparation:
(1) Wash potatoes clean and cut into small pieces.
(2) Boil for 3 or 4 hours under a free flame or in a steamer.
(3) Filter.
(4) Add 1.0 to 1.5% agar.
(5) Boil to dissolve agar and filter.
(6) Add 4.0% glycerol.
(7) Neutralize or may be used without neutralization.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

Reference: Lubinski (1895 p. 126).

2119. Ficker's Glycerol Potato Juice Agar

Constituents:

1. Potato juice	1000.0 cc.
2. Agar (1.0%)	10.0 g.
3. Glycerol (2.0%)	20.0 cc.

Preparation:
(1) Method of preparation of potato juice not given.
(2) Dissolve 1.0% agar in (1).
(3) Add 2.0% glycerol to (2).
(4) Reaction may be adjusted neutral, or slightly acid or alkaline.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli. The author reported that best growth was obtained on a neutral or slightly alkaline medium.

Variants: Klimmer gave the following method of preparation:

(1) Thoroly wash and scrub medium sized, sound unimpaired potatoes with a scrubbing brush, soap and water.
(2) Remove the peeling and wash in running tap water.
(3) Grind the potatoes to a pulp and press out the juice.
(4) Dissolve 40.0 parts glycerol and 15 parts agar in 1000 parts (3) by heating.
(5) Do not filter, clarify or neutralize.
(6) Tube.
(7) Sterilize (method not given).

References: Ficker (1900 p. 509), Klimmer (1923 p. 206).

2120. Weinzirl's Sucrose Potato Water Agar

Constituents:

1. Water	800.0 cc.
2. Potato water	200.0 cc.
3. Sucrose	10.0 g.
4. Agar	20.0 g.

Preparation:

(1) Mix 200.0 cc. of potato water and 800.0 cc. water.
(2) Dissolve 3 and 4 in (1).

Sterilization: Not specified.

Use: Cultivation of molds.

Reference: Weinzirl (1917 p. 441).

2121. Rochaix's Carrot Agar

Constituents:

1. Water	800.0 cc.
2. Carrot juice	200.0 cc.
3. Agar	30.0 to 35.0 g.

Preparation:

(1) Wash carrots well, chop and press out the juice.
(2) Dilute 200.0 cc. of the carrot juice with 800.0 cc. water.
(3) Add 30.0 to 35.0 g. of agar to (2).
(4) Heat in the autoclave for 20 to 25 minutes.
(5) Clarify by the addition of egg white.
(6) Make alkaline (indicator not specified).
(7) Filter thru paper.
(8) Distribute into tubes.

Sterilization: Sterilize at 108 or 110°. (Time not given.)

Use: General culture medium. The author reported that the addition of glycerol favored the growth of some organisms (tubercle bacilli).

Variants:

(a) The author added 10.0% glycerol to the agar.
(b) Harvey prepared a similar medium as follows:
    (1) Mince finely well washed carrots.
    (2) Press out the juice from the minced carrots.
    (3) Mix one part water with four parts carrot juice.
    (4) Add agar to 3.0%.
    (5) Steam for 45 minutes.
    (6) Clear with white of egg.
    (7) Adjust the reaction.
    (8) Filter.
    (9) Distribute into test tubes.
    (10) Sterilize.

References: Rochaix (1913 p. 604), Harvey (1921–22 p. 119).

2122. Schardinger's Hay Infusion Agar

Constituents:

1. Water	1000.0 cc.
2. Hay	30.0 to 40.0 g.
3. Agar (1.0 to 1.5%)	10.0 to 15.0 g.

Preparation:

(1) Boil 30.0 to 40.0 g. of hay with one liter of water.
(2) Filter.
(3) Add 1.0 to 1.5% agar to (2).
(4) Boil until agar is dissolved.
(5) Add Na_2CO_3 until the reaction is alkaline to litmus.

Sterilization: Method not given.

Use: Cultivation of protozoa. Wolbach and Binger cultivated *Spirocheta elusa* (free living) on a similarly prepared medium.

Variants: Wolbach and Binger mixed equal parts of an unheated filtered hay infusion, see medium 1179, and a melted and cooled 3.0% agar jelly.

References: Schardinger (1896 p. 541), Wolbach and Binger (1914 p. 10).

2123. Bordet and Gengou's Potato Blood Agar (Roos)

Constituents:

1. Distilled water	200.0 cc.
2. Glycerol (4.0%)	8.0 g.

3. Potato	100.0 g.
4. NaCl (0.65)	150.0 cc.
5. Agar	5.0 g.
6. Blood, defibrinated horse	

Preparation:

(1) Peel 100.0 g. potatoes, cut in small pieces and wash in running water for 2 hours.
(2) Add (1) to 200.0 cc. of distilled water containing 4.0% double distilled acid free glycerol.
(3) Autoclave for 40 minutes.
(4) Allow to stand over night and strain thru cheese cloth.
(5) Mix 50.0 cc. (4), 150.0 cc. of a 0.65% NaCl solution and 5.0 g. agar.
(6) Steam in the Arnold sterilizer until the agar is dissolved (30 to 60 minutes).
(7) Tube, do not filter.
(8) When ready for use, melt sterile tubes of (7), cool to about 45°C. and add the desired amount of sterile defibrinated horse blood.

Sterilization: Method of sterilization of agar not given.

Use: To maintain stock cultures of meningococci. Author reported that transplants must be made every two weeks. Incubate at 37.5°C. If tubes are paraffined fair growth after 6 weeks. Gonococci grow on this medium when blood content high and salt content diminished. Other authors cultivated the whooping cough bacillus, influenza bacillus, etc., on similar media.

Variants:

(a) Bordet and Gengou prepared the medium as indicated above. They distributed the agar in 2.0 or 3.0 cc. lots and added an equal volume of sterile defibrinated rabbit or human blood to the sterile agar.

(b) Bezançon prepared the medium as follows:
  (1) Add 100.0 g. of chopped potatoes to 200.0 cc. of a 4.0% glycerol solution and boil in the autoclave (time not given).
  (2) Separate the liquid from the potatoes (method not given).
  (3) Dissolve 6.0 g. NaCl and 50.0 g. of agar in a liter of water in the autoclave.
  (4) Add 50.0 cc. of the potato juice to 150.0 cc. of (3).
  (5) Tube in 2 to 3.0 cc. quantities.
  (6) Sterilize (method not given).
  (7) When ready for use melt the agar and add an equal quantity of whole or defibrinated human or rabbit blood. (Temperature of the agar not specified.)
  (8) Mix well.

(c) Harvey prepared a similar medium as follows:
  (1) Grate finely washed, peeled potatoes.
  (2) Add 100.0 g. to 100.0 cc. water.
  (3) Heat the mixture 20 minutes to a temperature not exceeding 50°C.
  (4) Raise to boiling temperature.
  (5) Boil 10 minutes.
  (6) Pour the mixture onto a clean, thick cloth.
  (7) Collect the fluid which drains thru the cloth together with that obtained by squeezing the cloth.
  (8) Filter the fluid collected thru thick, filter paper.
  (9) Add the filtrate to an equal quantity of distilled water.
  (10) Steam 60 minutes.
  (11) Add glycerol to 4.0%.
  (12) Mix well.
  (13) Filter.
  (14) Mix 10 parts (13) with 30 parts 0.6% NaCl solution and one part agar.
  (15) Sterilize.
  (16) Distribute into test tubes.

(d) Povitzky prepared the medium in the same manner as Bordet and Gengou using 2.0% glycerol as a final concentration instead of 1.0% and used defibrinated or citrated horse blood instead of defibrinated rabbit or human blood.

(e) Park, Williams and Krumwiede prepared the medium as follows:
  (1) Add 500.0 g. sliced potatoes and 80.0 cc. glycerol to 1000.0 cc. water.
  (2) Heat in the autoclave at 15 pounds pressure for 30 minutes.
  (3) Pour off the liquid.
  (4) To 500.0 cc. of the potato extract, add 1500.0 cc. of a 0.6% salt solution and 60.0 g. agar.

(5) Autoclave to dissolve.
(6) Filter.
(7) Tube.
(8) Sterilize in the autoclave for 30 minutes at 15 pounds pressure.
(9) When ready for use add an equal quantity of blood, or more often, one part blood to four parts agar.

References: Roos (1916 p. 68), Bordet and Gengou (1920 p. 734), Bezançon (1920 p. 121), Harvey (1921–22 p. 73), Klimmer (1923 p. 206), Povitzky (1923 p. 8), Park, Williams and Krumwiede (1924 p. 122).

2124. Schardinger's Straw Infusion Agar

Constituents:

1. Water	1000.0 cc.
2. Straw	30.0 to 40.0 g.
3. Agar (1.0 to 1.5%)	10.0 to 15.0 g.

Preparation:
(1) Same as medium 2122 but substituting straw for hay.

Sterilization: Method not given.

Use: Cultivation of protozoa and amoeba.

Variants:
(a) Tsujitani prepared a similar medium as follows:
  (1) Cut straw real fine.
  (2) Boil a mixture of 30.0 g. (1), 10.0 g. *Gigartina prolifera* (Tsunomato) and 1000.0 cc. water in a Koch steamer for one hour.
  (3) Filter thru a fine cloth.
  (4) Add 1.0% to 1.5% agar and 10.0 cc. normal soda solution.
  (5) Boil for 30 to 40 minutes.
  (6) Distribute into test tubes and sterilize (method not given).
(b) Harvey solidified 1000.0 cc. of a straw decoction with 15.0 g. agar. (See medium 1178 for the preparation of a straw decoction.)

References: Schardinger (1896 p. 541), Tsujitani (1898 p. 667), Harvey (1921–22 p. 121).

2125. Reed and Cooley's Spinach Agar

Constituents:

1. Water	500.0 cc.
2. Spinach	100.0 g.
3. Agar (2.0%)	10.0 g.

Preparation:
(1) Steep 100.0 g. of young spinach leaves in 500.0 cc. of water.
(2) After it is well steeped filter and add 2.0% agar.
(3) Dissolve the agar and filter thru cotton.

Sterilization: Sterilize by the intermittent method using the steamer.

Use: Cultivation of *Heterosporium variabile.* The authors reported that this was a good medium for the production of spores for the hanging drop cultures, since the fungus grew very short aerial hyphae.

Reference: Reed and Cooley (1911–12 p. 50).

2126. Owen's Cane Juice Agar

Constituents:

1. Cane juice (raw)	1000.0 cc.
2. Agar (2.0%)	20.0 g.

Preparation:
(1) Heat fresh raw cane juice.
(2) Filter thru cotton.
(3) Solidify with 2.0% agar.

Sterilization: Not specified.

Use: Bacterial count in cane sugar products.

Reference: Owen (1914 p. 337).

2127. Owen's Molasses Agar

Constituents:

1. Water	1000.0 cc.
2. Molasses (Final 75 Brix)	160.0 g.
3. Agar	20.0 g.

Preparation:
(1) Dissolve 160.0 g. final molasses 75 Brix and 20.0 g. agar in 1000.0 cc. of water.

Sterilization: Not specified.

Use: Bacterial count in cane sugar products.

Reference: Owen (1914 p. 338).

2128. Le Fevre and Round's Cucumber Juice Agar

Constituents:

1. Cucumber juice	1000.0 cc.
2. Agar (amount not given)	
3. NaCl	100.0 g.

Preparation:
(1) Method of obtaining cucumber juice not given.
(2) Dissolve 2 and 3 in (1).

Sterilization: Not specified.

Use: Cultivation and isolation of halophilic bacteria. Authors reported that organisms grew just as well on meat extract with 5.0% salt.

Reference: Le Fevre and Round (1919 p. 178).

2129. Bacto Lima Bean Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Lime bean infusion (dry)	8.0 g.
3. Agar, Bacto	15.0 g.

Preparation:

(1) Dissolve 23.0 g. of Bacto Lima Bean Agar (Dehydrated) in 1000.0 cc. of water, by boiling or autoclaving, preferably the latter. The dry lima bean infusion is prepared from an infusion of 50 parts dried lima beans.

(2) Distribute as desired.

Sterilization: Sterilize by autoclaving for 20 minutes at 15 pounds pressure.

Use: General culture medium.

Reference: Digestive Ferments Co. (1925 p. 17).

2130. Stutzer's Basal Legume Seed (Infusion) Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Legume seed	10.0 g
3. Agar	10.0 g.

Preparation:

(1) Same as for medium 1166, but solidified by the addition of 1.0% agar.

Sterilization: Not specified.

Use: Cultivation of nodule bacteria.

Variants: Zipfel prepared a similar medium as follows:

(1) Mix 100.0 g. of powdered legume seed with 100.0 cc. N/1 KOH in a mortar.

(2) Add 5 liters of water and allow to stand for 24 hours.

(3) Pour off the clear supernatant fluid.

(4) Neutralize with H_3PO_4 and make up to 5 liters volume.

(5) To 1 liter of (4) add 30.0 g. of agar and 20.0 g. dextrose.

(6) Acidify by the addition of 10.0 cc. N/1 malic acid.

(7) Sterilization not specified.

References: Stutzer (1900 p. 898), Zipfel (1911–12 p. 10).

2131. Wyant and Tweed's Pea Agar

Constituents:

1. Water	4000.0 cc.
2. Peas (canned)	1000.0 g.
3. Agar	60.0 g.
4. Litmus	

Preparation:

(1) Boil 3 in 1 until the agar is dissolved.

(2) Make up to water lost by boiling.

(3) Mash 1000.0 g. canned peas in their juice and add to (2).

(4) Autoclave for 30 minutes at 15 pounds pressure.

(5) Allow the agar to cool slowly. This allows the hulls to sink to the bottom.

(6) Cut away and discard the sediment after the agar has solidified.

(7) Melt the remaining clear agar and color with litmus.

Sterilization: Sterilize in the autoclave.

Use: Cultivation of organisms causing flat sour in peas.

Reference: Wyant and Tweed (1923 p. 12).

2132. Kaufmann's Jequirity Seed Agar

Constituents:

1. Water	100.0 cc.
2. Jequirity seeds	10.0 g.
3. Agar	1.5 to 2.0 g.

Preparation:

(1) Grind 10.0 g. Jequirity seeds in a mortar.

(2) The peeled or shelled seeds now weigh about 8.0 g.

(3) Add to 100.0 cc. of water.

(4) Boil in a steam sterilizer for about 2 hours.

(5) Cool and filter.

(6) Dissolve agar in (5).

(7) Reaction is neutral or slightly alkaline.

(8) Distribute into test tubes.

Sterilization: Sterilize in the usual manner (Method not given).

Use: Cultivation of *Bacillus pyocyaneus* and many others. Author reported that the medium supported the growth of a great variety of organisms.

Reference: Kaufmann (1891 p. 66).

2133. Maze's Sucrose Bean Agar

Constituents:

1. Water	1000.0 cc.

2. Beans (white)	
3. Sucrose (2.0%)	20.0 g.
4. NaCl (1.0%)	10.0 g.
5. Agar (1.5%)	15.0 g.

Preparation:

(1) Same as medium 1162 but solidified by the addition of 1.5% agar.

Sterilization: Not specified.

Use: To study nitrogen fixation by bacteria from nodules of leguminous plants.

Variants: de Rossi Gino prepared a similar medium as follows:

(1) Prepare agar using bean extract (method not given).
(2) Add 2.0% saccharose and 0.25% NaCl.
(3) Distribute in long necked flasks.
(4) Adjustment of reaction or sterilization not specified.

References: Mazé (1897 p. 45), de Rossi Gino (1909) (1910 p. 271).

2134. Reed and Cooley's Lima Bean Agar

Same as medium 2099, but *Phaseolus lunatus*, lima bean meal was used instead of corn meal.

2135. Thom's Bean Agar (Tanner)

Constituents:

1. Water	1000.0 cc.
2. Bean, white	200.0 g.
3. Agar	as desired

Preparation:

(1) Heat common white beans with 5 volumes of water. Boiling is stopped just before the swelling of the cotyledons rupture the seed coats.
(2) Filter. This solution filters easily.
(3) Add agar as desired.

Sterilization: Not specified.

Use: General culture medium. Tanner reported that this medium contained sufficient nutrients to grow many species normally. This medium is poor in available carbon. It may be desirable to add carbohydrates for certain species.

Reference: Tanner (1919 p. 50).

2136. Stutzer's Bean Infusion Agar

Same as variant (c), medium 1166, but solidified with 1.0% agar.

2137. Vogel and Zipfel's Legume Flour Agar (Klimmer)

Constituents:

1. Water	1000.0 cc.
2. Bean flour	50.0 g.
3. Agar	30.0 g.
4. Glucose	20.0 g.

Preparation:

(1) Add water to 50.0 g. of bean flour to make a thick paste.
(2) Dilute (1) to a liter by the addition of water.
(3) Allow to stand for 24 hours, shaking often.
(4) Remove the liquid from the sediment.
(5) Filter.
(6) Make up to one liter.
(7) Dissolve 30.0 g. agar and 20.0 g. glucose in (6).
(8) Do not adjust the reaction.

Sterilization: Not specified.

Use: Cultivation of nodule bacteria.

Variants: Klimmer used pea flour instead of bean flour.

Reference: Klimmer (1923 p. 229).

2138. Bacto Bean Pod Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Agar Bacto	15.0 g.
3. Bean Pod Meal Infusion (dry)	7.5 g.

Preparation:

(1) Dissolve 22.5 g. of Bacto Bean Pod Agar (Dehydrated) in 1000.0 cc. of distilled water by boiling or autoclaving, preferably the latter. Bean Pod Meal is prepared by infusion of 20 parts bean pod meal.
(2) Distribute as desired.

Sterilization: Heat in the autoclave for 20 minutes at 15 pounds pressure.

Use: General culture medium.

Reference: Digestive Ferments Co. (1925 p. 17).

2139. Didlake's Soy Bean Root Agar

Constituents:

1. Water	1000.0 cc.
2. Soy bean root	
3. Sucrose (5.0%)	5.0 g.

4. Asparagin (0.25%)	2.5 g.
5. Agar	

Preparation:

(1) Prepare a soy bean root infusion agar.

(2) Dissolve 0.5% sucrose and 0.25% asparagin in (1).

Sterilization: Not specified.

Use: Cultivation of a red pigment producing bacillus isolated from water. Author reported that the bacillus produced a red pigment only on this medium. Will grow more or less luxuriantly on soy bean agar leaving out the asparagin or sucrose, on ordinary beef peptone agar with 0.25% asparagin or 0.5% sucrose added or both asparagin and sucrose, or on a plain peptone water agar. On these media there was no pigment production.

Reference: Didlake (1905 p. 194).

2140. Zipfel's Legume Leaf Agar

Constituents:

1. Water	1000.0 cc.
2. Clover plant	1000.0 g.
3. Agar	30.0 g.
4. Glucose	20.0 g.

Preparation:

(1) Crush 1000.0 g. of closely picked clover leaves, (or other legume) to a pulp.

(2) Extract with hot water until finally 10 liters of liquid results.

(3) Filter the decoction when cold.

(4) Concentrate the filtrate to about 900.0 cc.

(5) Neutralize with N/1 NaOH.

(6) Make up to 1.0 liter.

(7) Distribute into small flasks. This is a concentrated stock solution.

(8) Dissolve 30.0 g. agar in 800.0 cc. water by heating.

(9) Add 20.0 g. glucose and 50.0 cc. of sterile (7) to (8).

(10) Adjust the reaction to neutral.

(11) Make up to 1 liter volume.

Sterilization: Sterilize (7) in the steamer. Sterilization of final medium not given.

Use: Cultivation of nodule bacteria.

Variants: The medium may be utilized with a neutral reaction, or add 10.0 cc. of N/1 malic or citric acid or 10.0 cc. of N/1 NaOH to give an alkaline medium.

Reference: Zipfel (1911–12 p. 106).

2141. Simon's Legume Agar (Klimmer and Krüger)

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. Legume infusion	2.0 cc.
4. Asparagin	10.0 g.
5. K_2CO_3	

Preparation:

(1) Prepare the legume infusion by pouring hot water over finely chopped straw of the legumes, mix well, macerate for two days and then boil an hour. Pour off the liquid, filter and evaporate until it contains 10.0% dry material.

(2) Dissolve 2 and 4 in 1000.0 cc. of water.

(3) Add a knife point of K_2CO_3 and 2.0 cc. of (1) to (2).

(4) Neutralize by the addition of NaOH using litmus as an indicator.

(5) Add malic acid to give a distinct acid reaction.

Sterilization: Not specified.

Use: Cultivation of nodule bacteria from leguminous plants.

Reference: Klimmer and Krüger (1914 p. 258), Klimmer (1923 p. 228).

SUBGROUP II-C. SECTION 9

Basal or complete media containing agar, with constituents of animal origin (exclusive of digests) of unknown chemical composition.

A_1. Containing animal products exclusive of extracts and infusions.

B_1. Cells, tissues or their derivatives used.

Teague and Deibert's Erythrocyte Agar 2142
Duval's Leprous Tissue Agar 2143
Nastiukoff's Egg Yolk Agar (Rechtsamer) 2144
Steinschneider's Egg Yolk Agar 2145
Brown's Albumen Agar (Giltner) 2146
Scales' Ammonium Lactate Egg Agar 2147
Scales' Egg Starch Agar 2148
Despeignes' Egg Yok Milk Agar 2149
Noyes' Gelatin Agar 2150
Standfuss and Kallert's Bone Jelly Agar 2151

B_2.* Body fluids used.

C_1. Blood employed.

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* See B_3, page 691.

Nicolle's Blood Agar 2152
Noguchi's Ringer Solution Plasma Agar (Abbott) 2153
C_2. Serous fluids employed.
Tanner's Serous Fluid Agar 2154
Kanthack and Stephens' Serous Exudate Agar 2155
Beck's Glycerol Serum Agar (Klimmer) 2156
C_3. Bile employed.
Ecker's Bile Agar 2157
B_3. Secretions, excretions or their derivatives used.
C_1. Milk or its derivatives used.
Raskin's Milk Egg Albumin Agar... 2158
Reinsch's Alkaline Milk Agar 2159
Abbott's Milk Agar 2160
Raskin's Casein Whey Agar 2161
Stutzer and Hartleb's Whey Agar.. 2162
Bronfenbrenner et al. Whey Agar... 2163
Ayres' Casein Agar (Tanner) 2164
Harvey's Nutrose Agar 2165
C_2. Milk or derivatives not used.
de Kruyff's Manure Agar 2166
A_2. Containing animal extracts or infusions.
B_1. Infusions specified.
C_1. Additional material, if any, inorganic.
D_1. Infusions of animal organs employed.
Henssen's Organ Infusion Agar 2167
Mayer's Salivary Gland Agar 2168
Graham-Smith's Heart Infusion Agar 2169
D_2. Other animal infusions employed.
Kutscher's Meat Infusion Agar 2170
Gassner's Basal Fuchsin Infusion Agar 2171
Harde and Hauser's Fish Infusion Agar 2172
Pergola's Mussel Infusion Agar 2173
C_2. Containing additional organic material.
D_1. All additional constituents of known chemical composition.
E_1. Organic nitrogen supplied
E_2. Organic nitrogen not added.
F_1. Carbohydrates supplied.
Harvey's Glucose Starch Infusion Agar 2174
Vedder's Starch Infusion Agar 2175
F_2. Alcohols added.
Kopp's Thyroid Agar 2176
Wroblewski's Suprarenal Capsule Agar 2177
Ficker's Brain Agar 2178
Ficker's Spleen Agar 2179
Duval's Turtle Agar 2180
Loeffler's Malachite Green Nutrose Agar 2181
D_2. Containing additional materials of unknown chemical composition.
Meyers' Tissue Agar 2182
Williams and Burdick's Egg Veal Agar 2183
B_2. Extracts specified.
C_1. Additional material, if any, inorganic. (Exclusive of indicators).
Bürgers' Basal Lead Acetate Agar.. 2184
Hiss' Extract Agar 2185
C_2. Containing additional organic materials.
D_1. All additional organic constituents of known chemical composition.
Bacto Starch Agar (Dehydrated)... 2186
Hiss' Glucose Extract Agar 2187
Tanner's Malachite Green Extract Agar 2188
Noyes' Starch Extract Agar 2189
Malenkovic's Xylose Extract Agar. 2190
D_2. Containing additional organic constituents of unknown chemical composition.
Hiss' Gelatin Extract Agar 2191
Stapp's Egg White Extract Agar... 2192

2142. Teague and Deibert's Erythrocyte Agar

Constituents:
1. Distilled water.
2. NaCl.
3. Red Blood Cells.
4. Agar.

Preparation:
(1) Add 2.0 cc. of red blood cells (obtained by centrifuging defibrinated blood and removing the serum) to 10.0 cc. of normal NaCl solution.
(2) Keep the temperature at 100°C. for 3 minutes.
(3) Shake the tubes and allow to cool.
(4) Centrifuge and obtain the supernatant fluid.
(5) Add various amounts of (4) to a 2.0% water solution containing 0.5% NaCl.

Sterilization: Method not given.

Use: To study growth requirements of Unna-Ducrey bacillus. The authors reported that a fair growth occurred when rabbit serum was added, but no growth using sheep serum, or without the addition of serum.

Variants: The authors added various amounts of sheep or rabbit serum, heated at 55°C. for 15 minutes to the medium as prepared above.

Reference: Teague and Deibert (1922 p. 70).

2143. Duval's Leprous Tissue Agar

Constituents:

1. Agar solution.
2. Tissue (Leprous).

Preparation:

(1) Prepare an agar solution. Composition not given, but the author specified that it should contain no peptone.

(2) Adjust to 1.5% alkaline.

(3) Place small bits of leprous tissue upon the surface of the sterilized and solidified alkaline agar.

(4) Moist the surface with a thin suspension of an organism capable of oxidizing nucleo-proteids such as *B. typhosus*, *paratyphosus*, *prodigiosus*, *pyocyaneus*, *dysenteriae*, etc.

(5) Incubate at 32°C. to 35°C. for two weeks.

Sterilization: Sterilization of agar not given.

Use: Indirect culture of *B. leprae*.

Reference: Duval (1911 p. 371).

2144. Nastiukoff's Egg Yolk Agar (Rechtsamer)

Constituents:

1. Distilled water	1000.0 cc.
2. Egg yolk	100.0 cc.
3. Agar	15.0 to 20.0 g.

Preparation:

(1) Separate the yolk of egg from the white by Bunge's method (allow the whites to roll from a blotting paper containing the egg).

(2) Add 0.5 cc. of a 10.0% NaOH solution and 100.0 cc. of egg yolk to 1000.0 cc. distilled water.

(3) Add 15.0 to 20.0 g. of agar to (2) and boil until there is complete solution of the agar.

(4) Allow to stand two hours in the steamer.

(5) Filter the thick liquid thru a hot water funnel.

(6) Distribute into sterile test tubes.

Sterilization: Sterilize in the steamer.

Use: Cultivation of diphtheria, cholera, influenza and other pathogenic forms.

References: Nastiukoff (1893 #33 and 34), Rechtsamer (1895 p. 493).

2145. Steinschneider's Egg Yolk Agar

Constituents:

1. Distilled water	800.0 cc.
2. Egg yolk	100.0 cc.
3. Agar (2.0% soln.)	

Preparation:

(1) Mix two parts of sterile distilled water with one part hen or lapwing's egg yolk obtained under sterile conditions.

(2) Prepare a 2.0% agar solution.

(3) Mix one part (1) with two parts (2).

(4) Distribute into tubes.

(5) Slant and solidify.

Sterilization: Final sterilization not given.

Use: Cultivation of gonococcus. The medium was not transparent.

Variants: The author prepared a similar medium as follows:

(1) Mix one part yolk of hen or lapwing's egg with three parts sterile distilled water. Shake thoroly.

(2) To 20.0 g. of (1) add 10.0 cc. of a 20.0% Na_2HPO_4 and 3 times, hence 90.0 cc. of a 2.5 to 3.0% agar solution.

(3) Pour into tubes and solidify (method not given).

(4) Final sterilization not specified.

The author reported that this medium was not yellow and was nearly transparent. Gonococcus growth was not quite so luxuriant on this medium as on serum agar.

Reference: Steinschneider (1897 p. 380).

2146. Brown's Albumin Agar (Giltner)

Constituents:

1. Distilled water	1000.0 cc.
2. K_2HPO_4	0.5 g.
3. $MgSO_4$	0.2 g.
4. Egg albumin (powdered)	0.1 g.
5. Glucose	10.0 g.
6. $Fe_2(SO_4)_3$	trace
7. Agar	15.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, and 7 in 1.

(2) Do not filter.

Sterilization: Not specified.

Use: To determine the bacterial count of soil. See medium 1439.

Reference: Giltner (1921 p. 373).

2147. Scales' Ammonium Lactate Egg Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	15.0 g.
3. Na_2HPO_4	2.0 g.
4. Ammonium lactate	10.0 cc.
5. $CaCO_3$	10.0 g.
6. Egg	

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Titrate to +3.
(3) Dissolve 4 and 5 in (2).
(4) Add 2.0 cc. of whole egg to 7.0 cc. of (3).
(5) Tube.

Sterilization: Sterilize at 12 pounds pressure for 15 minutes.

Use: To study variation in morphology of *B. coli*.

Reference: Scales (1921 p. 595).

2148. Scales' Egg Starch Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Starch (soluble 0.1%)	1.0 g.
3. Agar (1.5%)	15.0 g.
4. Eggs	

Preparation:

(1) Strain a fresh whole egg thru four thicknesses of cheese cloth four times.
(2) Prepare a soluble solution of agar 1.5%, and 0.1% soluble starch in distilled water
(3) Add 2.0 cc. of (1) to each 7.0 cc. of (2).

Sterilization: Sterilize at 12 pounds pressure for 15 minutes.

Use: To study variations in morphology of *B. coli*.

Reference: Scales (1921 p. 596).

2149. Despeignes' Egg Yolk Milk Agar

Constituents:

1. Distilled water	
2. Milk	100.0 cc.
3. Glycerol	15.0 g.
4. Agar	9.0 g.
5. Gentian violet	
6. Egg Yolk	

Preparation:

(1) Add 100.0 cc. of boiled skim milk, 15.0 g. glycerol and 9.0 g. of agar to 200.0 cc. of Besredka's egg yolk solution (see variant of medium 1244)
(2) Heat in a salt water bath until the agar is dissolved.
(3) Sterilize in the autoclave at 105°C. (Time not specified.)
(4) Add 3.0 cc. of 1.0% gentian violet solution when cool.
(5) Distribute in sterile tubes under aseptic conditions.
(6) Slant.

Sterilization: Heat on 3 successive days at 55 to 57°C. for 30 minutes.

Use: Isolation tubercle bacilli.

Reference: Despeignes (1922 p. 120).

2150. Noyes' Gelatin Agar

Constituents:

1. Water	1000.0 cc.
2. Agar (best)	15.0 g.
3. Gelatin	7.5 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: Cultivation of soil bacteria. Weiss used a similar medium to determine the bacterial count of water.

Variants:

(a) Noyes added 1.0 or 2.0 g. starch.
(b) Weiss prepared a similar medium as follows:
  (1) Dissolve 15.0% gelatin in 500.0 cc. of water from the "Mühlgraben der Schwarzawa."
  (2) Dissolve 1.5% agar in 500.0 cc. water.
  (3) Mix (1) and (2).

References: Noyes (1916 p. 93), Weiss (1920 p. 25).

2151. Standfuss and Kallert's Bone Jelly Agar

Solidify medium 1360 with agar.

2152. Nicolle's Blood Agar

Constituents:

1. Water	900.0 cc.
2. Agar	14.0 g.
3. NaCl	6.0 g.
4. Blood, rabbit	

Preparation:

(1) Dissolve 2 and 3 in 1.
(2) Neutralize or make slightly alkaline.
(3) Distribute in tubes of the same diameter.
(4) Cool sterile (3) to 48 to 52°C. and add one third the volume of rabbit blood to each tube. Draw the blood directly from the heart under aseptic conditions.
(5) Slant the tubes.

Sterilization: Sterilize (3) in the autoclave.

Use: Cultivation of kala azar parasite. Similar media were used for the cultivation of *Prioplasma donovai*, *Piroplasma equi*, other piroplasma, trypanosomes, leishmania, and protozoa.

Variants:

(a) Harvey cultivated trypanosomes on a medium prepared as follows:
  (1) Dissolve 0.6 g. NaCl and 1.5 g. agar in 100.0 cc. water.
  (2) Mix equal parts of (1) and defibrinated rabbit blood at 45°C.
(b) Harvey prepared a N. N. N. or whole blood agar as follows for the cultivation of leishmania:
  (1) Dissolve 6.0 g. NaCl and 16.0 g. of well washed agar in 900.0 cc. of water.
  (2) Sterilize (1), method not given.
  (3) Mix one part rabbit blood with 3 parts (2) at 50°C.
  (4) Slope.
  (5) Test sterility.
  (6) Preserve in the dark.
(c) Harvey cultivated the trypanosomes of cold blooded animals on the following medium:
  (1) Dissolve 20.0 g. of agar in 1000.0 cc. tap water.
  (2) Tube and sterilize.
  (3) Mix equal parts (2) and sterile defibrinated rabbit blood.
  (4) Solidify in the sloped position and inoculate in the water of condensation.
(d) Leiva cultivated *Leishmania infantum* and *Leishmania ctenocephali* on a medium prepared as follows:
  (1) Dissolve 20.0 g. agar and 4.0 g. NaCl in 1000.0 cc. water. (May use 5.0 g. agar.)
  (2) Tube.
  (3) Add 25.0% (Use only 5.0% when using 0.5% agar) sterile defibrinated rabbit blood to sterile melted and cooled (2).
(e) Stitt gave the following method of preparation of N. N. N. medium for the cultivation of trypanosomes, leishmania and protozoa:
  (1) Dissolve 14.0 g. agar and 6.0 g. NaCl in 900.0 cc. of water.
  (2) Tube and sterilize.
  (3) To one part of (2) liquified and cooled to 48°C. add one third its volume of defibrinated rabbit blood. (Use human or rat blood for the cultivation of human trypanosomes.)
  (4) Mix thoroly.
  (5) Slant.
  (6) Use rubber stoppers or cover cotton plugs with paraffin.

References: Nicolle (1909 p. 397), Marzinowsky (1908–09 p. 419). Bezançon (1920 p. 120), Harvey (1921–22 pp. 72, 74), Leiva (1922 p. 179) taken from (1923 p. 342), Stitt (1923 p. 52).

2153. Noguchi's Ringer Solution Plasma Agar (Abbott)

Constituents:

1. Water	1000.0 cc.
2. NaCl	10.0 g.
3. KCl	0.2 g.
4. $CaCl_2$	0.2 g.
5. $NaHCO_3$	0.1 g.
6. Glucose	1.0 g.
7. Serum, rabbit	
8. Citrate plasma	
9. Agar (2.0%)	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1. (Ringer solution.)
(2) Add 1.5 parts serum and 1 part citrate plasma to 4.5 parts (1).
3) Stiffen the medium by the addition of "sterile agar (free of peptone and sugar) in the proportion of 2.0%."

Sterilization: Not specified.

Use: Cultivation of spirochaetes.

Variants:

(a) Kaneko cultivated *Spirochaeta icterohaemorrhagiae* and *Spirochaeta hebdomadis* on a medium prepared as follows:

(1) Dissolve 3.0 g. of agar in 100.0 cc. Ringer's solution.
(2) Add a drop of dog blood to 2 to 3.0 cc. of dog serum diluted with 2 to 5 parts Ringer's solution. (Sera or ascitic fluids of other animals may be used.)
(3) To 9.0 cc. of (2) add 1.0 cc. of (1).
(4) Heat at 56 to 58°C. in a water bath for about 30 minutes.
(5) Cover with a layer of liquid sterile paraffin.

(b) Stitt cultivated *Leptospira icteroides* (cause of yellow fever) on a medium prepared as follows:
(1) Mix one part rabbit serum and 3 parts of Ringer's solution solidified partially by the addition of 0.3% agar.
(2) Tube in tall tubes.
(3) Introduce 1.0 cc. of yellow fever patients blood into the lower part of each tube.
(4) Pour a thin layer of petroleum over the top of the medium.

References: Abbott (1921 p. 635), Kaneko (1921–22 p. 354), Stitt (1923 p. 54).

2154. Tanner's Serous Fluid Agar

Constituents:

1. Distilled water	100.0 cc.
2. Agar	1.5 g.
3. Serum	100.0 cc.

Preparation:
(1) Dissolve 1.5 g. agar in 100.0 cc. distilled water.
(2) Filter and tube in 5.0 cc. quantities.
(3) Add an equal volume of sterile serum to each tube of sterile (2) melted and cooled to 40°C.
(4) Roll the tube to mix.
(5) Cool in a slanting position.

Sterilization: Method of sterilization of (2) not given.

Use: General culture medium. Teague and Deibert used a similar medium to study growth requirements of Unna-Ducrey bacillus.

Variants:
(a) Harvey specified that the agar be sterilized at 120°C., and be mixed with an equal volume of sterile serum or ascitic fluid.
(b) Teague and Deibert reported that the Unna-Ducrey bacillus would not grow on a medium prepared by mixing various amounts of sheep or rabbit serum, heated at 55°C. for 15 minutes with a 2.0% agar solution containing 0.5% NaCl.

References: Tanner (1919 p. 70), Harvey (1921–22 p. 79), Teague and Deibert (1922 p. 70).

2155. Kanthack and Stephens' Serous Exudate Agar

Constituents:

1. Serous exudate (serum, ascitic fluid, etc.)	100.0 cc.
2. KOH 10.0%	2.0 cc.
3. Agar (1.5 to 2.0%)	15.0 to 20.0 g.
4. Glycerol (4.0 to 5.0%)	4.0 to 5.0 cc.

Preparation:
(1) To 100.0 cc. of a serous exudate add 2.0 cc. of 10% KOH solution. (It is always best to first heat a small portion of the serous exudate in a test tube. If it solidifies or if a large amount of albumin appears, add at least a double volume of distilled water before adding KOH.)
(2) Soak agar in acidified water and wash free from acid.
(3) Add 1.5 to 2.0% agar to (1).
(4) Boil until the agar is completely dissolved. (The Mixture appears clear when agar is dissolved.)
(5) Filter thru a coarse filter paper using a hot water funnel.
(6) Add 4.0 to 5.0% glycerol.
(7) Distribute into test tubes.

Sterilization: Not specified.

Use: Diagnosis of diphtheria. The authors reported that staphylococci and *B. coli communis*, etc., development was inhibited. The sooner the exudate was used, after it left the human body the clearer the medium.

Variants:
(a) The authors reported that the addition of 2.0 to 4.0% glucose did not improve the medium.
(b) Harvey prepared a similar medium as follows:
(1) Collect ascitic fluid with sterile precautions.

(2) Leave overnight to allow for separation of clot.
(3) Add 50.0 cc. N/1 sodium hydroxide per liter.
(4) Steam 20 minutes.
(5) Add 1.0 g. agar per liter after making it into a paste or suspension with a little of the alkaline ascitic fluid.
(6) Steam to dissolve the agar.
(7) Filter, while hot, thru thick, filter paper by placing filter funnel, stand and receptacle for filtrate in the sterilizer.
(8) Dissolve 10.0 g. glucose per liter in the hot, filtered, nutrient agar (7). (Add 50.0 cc. glycerol per liter also if desired.)
(9) Distribute into test tubes.
(10) Sterilize.

(c) Harvey prepared a similar medium from 20.0 g. glycerol, 20.0 cc. of 10.0% NaOH, 20.0 g. agar and 1000.0 cc. of a body fluid (ascitic fluid, pleuritic fluid, hydrocele fluid, ovarian fluid, milk, urine, etc.).

References: Kanthack and Stephens (1896 p. 609), (1896 p. 835), Harvey (1921–22 p. 83).

2156. Beck's Glycerol Serum Agar
(Klimmer)

Constituents:

1. Water	1800.0 cc.
2. Serum (beef)	200.0 cc.
3. $KHSO_4$	10.0 g.
4. $MgSO_4$	5.0 g.
5. Asparagin	4.0 g.
6. Glycerol	40.0 g.
7. Agar (3.0%)	60.0 g.

Preparation:
(1) Steam 200.0 cc. beef serum (containing no chloroform) with 1800.0 cc. of water for 1.0 to 1.5 hours.
(2) Filter.
(3) Add 3, 4, 5 and 6 to the filtrate and steam for 2 to 3 hours.
(4) Filter while hot.
(5) Add 3.0% fiber agar, and heat until dissolved.
(6) Tube.
(7) Reaction should be slightly acid.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

Reference: Klimmer (1923 p. 224).

2157. Ecker's Bile Agar

Constituents:

1. Bile (ox)	1000.0 cc.
2. Agar	15.0 g.

Preparation:
(1) Dissolve 2 in 1 as rapidly as possible by gently boiling.
(2) Filter and allow to cool and again boil and refilter without titration.
(3) Tube.

Sterilization: Sterilize in autoclave for 3 minutes at 15 pounds pressure.

Use: To study effect of bile on *B. typhosus*. The author reported that some strains were completely inhibited while others were only partially inhibited. Approximately the same results were obtained using 50.0% or 10.0% bile. (Bile diluted with distilled water.)

Reference: Ecker (1918 p. 97).

2158. Raskin's Milk Egg Albumin Agar

Constituents:

1. Milk	1000.0 cc.
2. Egg albumin	
3. NaCl	5.0 g.
4. Agar	5.0 to 7.0 g.

Preparation:
(1) Stir thoroughly with a glass rod 100.0 g. albumin from fresh eggs in a flat bottomed dish and add drop by drop concentrated NaOH until a solid opaque gelatinous material is obtained.
(2) Cut this solid egg albumin in small pieces and place in distilled water. Do not allow the alkaline albuminate to stand or the albuminate will become liquid in several hours.
(3) Shake thoroughly and pour off the water, continue this washing until the wash water is only slightly alkaline.
(4) Place the coagulated sodium albuminate in a flask of distilled water, plug with cotton and heat in the steamer for 15 to 30 minutes. The albumin dissolves.
(5) Filter.
(6) Add 50.0 cc. glycerol and 5.0 to 7.0 g of finely divided dried agar to 1000.0 cc. of fresh milk.
(7) Allow to stand for 12 to 14 hours (at room temperature in the winter) and then boil for 75 to 90 minutes

over a free flame. In order to prevent loss of water during boiling place a lid over the container or steam for 3 to 3.5 hours in a steamer. The casein coagulates slowly.

(8) Press thru four folds of linen cloth.
(9) The reaction of the fluid is slightly acid.
(10) Pour into a tall glass cylinder and place in a warm thermostat.
(11) After about 20 or 30 minutes remove the yellow top layer containing the fat by means of a spoon.
(12) Heat the fat-free portion to boiling and add 1.0% of the filtrate from (5).
(13) Add soda to neutralize the reaction.
(14) Add 0.5% NaCl if desired.
(15) Filter until clear thru a paper in a hot water funnel.

Sterilization: Not specified.

Use: Cultivation of pathogenic organisms.

Reference: Raskin (1887 p. 359).

2159. Reinsch's Alkaline Milk Agar

Constituents:

1. Water	100.0 cc.
2. Agar	3.0 to 4.0 g.
3. Milk	500.0 cc.
4. NaOH	1.0 g.

Preparation:

(1) Soak 3.0 to 4.0 g. of powdered agar in 100.0 cc. water for 24 hours.
(2) Add egg white and boil for 3 to 4 hours in a steamer.
(3) Filter.
(4) Place 500.0 cc. of fresh cow milk in a separatory funnel and add 1.0 g. NaOH (2.5 cc. solution of 400.0 g. NaOH in a liter).
(5) Shake well and allow to stand at about 18°C. for 48 hours.
(6) Remove the nearly transparent milk from the bottom of the funnel and add it to a second separatory funnel.
(7) Add 250.0 cc. ether and shake well.
(8) Allow to stand for 48 hours.
(9) Place the opalescent liquid now in a large sterile flask, plug with cotton and heat to 50°C.
(10) Place under the receiver of a water suction pump for 3 or 4 hours until all the ether is evaporated.
(11) Mix 1 part of (3) with 3 parts of (10) at about 50°C.
(12) Distribute into sterile test tubes.
(13) Incubate to test sterility.

Sterilization: Sterilization of (3) not given.

Use: Cultivation of typhoid bacilli and others. The author reported that the agar was light yellow, transparent and slightly opalescent.

Variants: The author prepared a slightly darker medium as follows:

(1) Place 500.0 cc. of fresh cow milk in a separatory funnel and add 1.0 g. NaOH (2.5 cc. solution of 400.0 g. NaOH in a liter).
(2) Shake well and allow to stand at about 18°C. for 48 hours.
(3) Remove the nearly transparent milk from the bottom of the funnel and add it to a second separatory funnel.
(4) Add 250.0 cc. ether and shake well.
(5) Allow to stand for 48 hours.
(6) Place the opalescent liquid now in a large sterile flask plug with cotton and heat to 50°C.
(7) Place under the receiver of a water suction pump for 3 or 4 hours until all the ether is evaporated.
(8) Add 1.5% pulverized agar.
(9) Digest for 24 hours at room temperatures.
(10) Heat in steamer for 2–3 hours and filter.

Reference: Reinsch (1892 p. 31).

2160. Abbott's Milk Agar

Constituents:

1. Milk	1000.0 cc.
2. Agar (1.0 to 1.5%)	10.0 to 15.0 g.

Preparation:

(1) Solidify cream-free milk by the addition of 1.0 to 1.5% agar.

Sterilization: Not specified.

Use: General culture medium.

Reference: Abbott (1921 p. 139).

2161. Raskin's Casein Whey Agar

Constituents:

1. Milk	1000.0 cc.
2. Agar	

Preparation:

(1) Allow 1000.0 cc. of skimmed (or whole) milk to stand 48 hours at room temperature.
(2) Remove any cream that settles out and heat the sour milk at 70 to 80°C.

(3) Obtain the casein and thoroly press it dry.
(4) Rub the casein to a powder and wash with 95.0% alcohol, and place in a flask of ether.
(5) Shake for 2 minutes and allow to stand for 18 minutes.
(6) Pour off the ether and add fresh. Repeat this process 3 or 4 times.
(7) Pour off but half the last portion of the ether and add alcohol (amount not specified).
(8) Shake for 5 minutes.
(9) If drops of fat raise when alcohol is added, repeat the washing of the casein with ether until the casein is fat-free.
(10) Collect the casein on a filter.
(11) Dry and heat for 5 to 20 minutes at 120 to 140°C.
(12) Wash in a moderately concentrated alkali solution.
(13) This gives a transparent material, which when dry becomes hard as stone. This casein will dissolve by heating in slightly alkaline water.
(14) Prepare 150.0 cc. of an 8.0% solution of (13).
(15) Mix 350.0 cc. of filtered whey containing 1.75% agar with (14).
(16) Heat for 15 to 20 minutes at 60 or 70°C. Do not boil or the casein will be coagulated.
(17) Distribute in sterile test tubes.

Sterilization: Not specified.

Use: Cultivation or pathogenic organisms.

References: Raskin (1887 p. 359), Peifer (1888 p. 568).

2162. Stutzer and Hartleb's Whey Agar

Constituents:

1. Whey	1000.0 cc.
2. Agar	20.0 g.

Preparation:

(1) Separate the cream from milk by centrifugation.
(2) Heat to 40°C. and add a little rennet.
(3) After the casein has coagulated heat to 75°C. and filter.
(4) To a liter of (3) add 20.0 g. of finely chopped agar and heat in the steamer for 1.5 to 2.0 hours.
(5) Neutralize by adding soda (indicator not specified).
(6) Medium may be employed with a neutral, basic, (by adding Na_2CO_3), or acid, (by adding lactic acid), reaction.

Sterilization: Not specified.

Use: Stutzer and Hartleb attempted to cultivate the bacterium causing foot and mouth disease. Other investigators used similar media for the cultivation of a large number of organisms.

Variants:

(a) Huss cultivated aroma producing bacteria, *Bacillus esterificans* and *Pseudomonas trifolii* on a medium prepared by dissolving 20.0 g. agar and 5.0 g. NaCl in 1000.0 cc. of whey.

(b) Meier made bacterial counts of milk and milk products in a medium prepared as follows:
  (1) Dissolve 2.5 g. NaCl and 15.0 or (10.0 g.) agar in 500.0 cc. of water.
  (2) Mix equal parts (1) and whey.
  (3) Neutralize by the addition of KOH. Add KOH until tumeric paper is turned quite weakly brownish-red.
  (4) Sterilization not specified.

(c) Fulmer and Grimes used the following medium for the cultivation of yeast found in cream and butter.
  (1) Dissolve 1.5% agar in whey obtained from skim milk by coagulating the casein with rennet.
  (2) Filter (1) thru absorbent cotton.
  (3) Tube in 10.0 cc. portions.
  (4) Sterilize for 20 minutes at 15 pounds pressure.
  (5) When using as a plate medium, add 1.0 cc. of a 1.0% tartaric acid solution to each Petri dish to keep down bacterial growth.

(d) Klimmer and Sommerfeld (Klimmer) made bacterial counts of milk on a medium prepared as follows:
  (1) Prepare clear agar solution using 20.0 g. agar, 7.0 g. NaCl and 1000.0 cc. water.
  (2) Sterilize (1) method not given.
  (3) Add a little rennet to milk obtained under the cleanest possible conditions and heat at 40°C. until the casein is coagulated.
  (4) Separate the casein from the whey by straining thru a straining cloth.

(5) Filter the whey thru filter paper and then thru an asbestos filter.
(6) Distribute in sterile flasks.
(7) Sterilize by the addition of 1.5% chloroform.
(8) Pour in plates.

(e) Klimmer solidified whey, prepared from milk by coagulating the casein at 40°C. in the presence of rennet, with agar.

References: Stutzer and Hartleb (1897 p. 403), Huss (1907 p. 58), Meier (1918 p. 435), Fulmer and Grimes (1923 p. 585), Klimmer (1923 pp. 172, 203).

2163. Bronfenbrenner et al. Whey Agar

Constituents:

1. Water	200.0 cc.
2. Milk	100.0 cc.
3. CR indicator	1.5 cc.
4. Agar (3.0%)	6.0 g.

Preparation:

(1) Use fresh milk. Syphon the milk from under the cream.
(2) Bring it to boiling and add 2.5 cubic centimeters of 10.0% $MnCl_2$ solution to each 100 cubic centimeters of milk.
(3) Cool the mixture as soon as clot is formed and filter thru a single layer of cloth.
(4) Titrate an aliquot portion hot and adjust the bulk of medium to neutral reaction (1×10^{-7}).
(5) Bring quickly to boiling, cool and filter thru paper.
(6) At this time prepare an agar jell of 3.0% concentration in plain water, sterilize and dilute with an equal volume of neutralized milk-whey.
(7) While the mixture is hot add CR indicator at the rate of 0.5 cubic centimeters for each 100 cubic centimeters of medium distributed into sterile tubes.

Sterilization: Method of sterilization of agar solution (see step (6) above) not given. Sterilize the final medium for 10 minutes at 15 pounds pressure.

Use: Cultivation of colon-typhoid group. Authors reported that the medium gave as good results as lactose peptone agar as a medium for colon-typhoid bacteria and was cheaper and more easily prepared.

Reference: Bronfenbrenner, Davis and Morishima (1918–19 p. 347).

2164. Ayres' Casein Agar (Tanner)

Constituents:

1. Water	1000.0 cc.
2. Casein	10.0 g.
3. NaOH (N/1)	7.0 cc.
4. Agar	10.0 g.

Preparation:

(1) Add 10.0 g. casein (Eimer and Amend C.P. casein prepared according to Hammersten) and 7.0 cc. of normal NaOH to 300.0 cc. of water.
(2) Boil until solution is complete. It is desirable to allow the mixture to stand several hours.
(3) Make up to 500.0 cc. volume.
(4) Bring the reaction between +0.1 and +0.2 Fuller's scale. Do not allow the solution to become alkaline to phenolphthalein or over +0.2.
(5) Filter.
(6) Dissolve 10.0 g. agar in 500.0 cc. of water.
(7) Filter.
(8) Mix (5) and (7).
(9) Tube.

Sterilization: Sterilize in the autoclave under pressure for 20 minutes and cool the tubes in cold water or ice water.

Use: General culture medium. Harvey cultivated soil bacteria on a medium prepared in a similar manner.

Variants:

(a) Harvey prepared the medium as follows:
  (1) Mix 10.0 g. casein with 100.0 cc. distilled water.
  (2) Add N/1 sodium hydroxide 7.0 cc.
  (3) Steam until the casein is dissolved.
  (4) Dissolve 10.0 g. agar in 900.0 cc. distilled water.
  (5) Mix the agar solution with the casein solution.
  (6) Filter thru thick filter paper.
  (7) Adjust the reaction to 1.5% acid to phenolphthalein.
  (8) Distribute into test tubes.
  (9) Sterilize in autoclave.

Reference: Tanner (1919 p. 70), Harvey (1921–22 p. 96).

2165. Harvey's Nutrose Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	15.0 g.
3. Nutrose	15.0 g.

Preparation:

(1) Dissolve 15.0 g. of nutrose (or somatose) and 15.0 g. agar in 1000.0 cc. of water.

Sterilization: Not specified.

Use: Cultivation of amoebae.

Reference: Harvey (1921–22 p. 97).

2166. de Kruyff's Manure Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	20.0 g.
3. K_2HPO_4	0.1 g.
4. NH_4NO_3	0.5 g.
5. Stable manure	50.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1. Details of method of preparation not given).

Sterilization: Not specified.

Use: Cultivation of *Myxococcus javanensis*.

Reference: de Kruyff (1908 p. 385).

2167. Henssen's Organ Infusion Agar

Constituents:

1. Water	200.0 cc.
2. Kidney	100.0 g.
3. Agar	2.5 g.

Preparation:

(1) Remove the capsule from the kidney (dog, calf and hog were used) and rub to a pulp in a mortar with an equal amount of water.

(2) Allow to extract for 3 hours.

(3) Press thru fine pored linen.

(4) Prepare a 2½% water solution of agar.

(5) Heat (4) to 40°C.

(6) Mix equal parts of sterile (5) and sterile melted agar cooled to 40°C.

(7) Incubate to test sterility.

Sterilization: Filter (3) thru a sterile clay filter using a water suction pump to facilitate the filtering. Method of sterilization of agar not given.

Use: To study the effect of kidney infusion on the growth of some organisms. The author reported that the medium checked the growth of the organisms studied, i.e., diphtheria bacilli, anthrax bacilli, *B. coli*, typhoid and cholera organisms. Livingood used a similar medium using *B. coli*, *B. typhosus*, *B. anthracis*, *B. diphtheriae*, *B. pseudodiphtheria*, and reported that liver gave the best general results.

Variants: Livingood prepared a similar medium as follows:

(1) Pass 1 pound of organs (liver, beef and hog, or spleen, sheep and hog, or or adrenals, beef and sheep) thru a meat chopper and macerate with 1000.0 cc. of tap water for 12 hours on ice.

(2) Press the juice thru a sterile towel.

(3) Force the fluid thru a Chamberland filter using carbonic acid pressure and collect in a sterile flask.

(4) Prepare a 2.0% agar solution (no NaCl or peptone) sterilize. (Bouillon agar, with NaCl and peptone, may be used.)

(5) Mix equal parts of melted (4) and (3).

(6) Incubate for 24 to 48 hours to determine sterility.

References: Henssen (1895 p. 403), Livingood (1898 p. 981).

2168. Mayer's Salivary Gland Agar

Constituents:

1. Water	500.0 cc.
2. Salivary glands	500.0 g.
3. Agar (1.5%)	15.0 g.

Preparation:

(1) Chop fresh salivary glands in a meat chopping machine.

(2) Mix with an equal weight of water.

(3) Infuse on ice for 24 hours after strong stirring.

(4) Press the mass in a pressing machine.

(5) Solidify sterile (6) by the addition of 1.5% agar (method not given).

Sterilization: Sterilize (4) in streaming steam for 30 minutes. Final sterilization not specified.

Use: General culture medium. Author reported that meat extract media generally gave better results.

Reference: Mayer (1899 p. 747).

2169. Graham-Smith's Heart Infusion Agar

Solidify medium 1342 by the addition of agar.

2170. Kutscher's Meat Infusion Agar

Constituents:

1. Meat water	1000.0 cc.
2. Agar (1.5%)	15.0 g.

Preparation:

(1) Prepare meat water.
(2) Dissolve 15.0 g. agar in sterile (1) by boiling over a free flame.
(3) Make slightly alkaline by the addition of soda solution.
(4) Filter.

Sterilization: Method of sterilization of meat water not given. Method of sterilization of the final medium not given.

Use: Isolation and cultivation of *Spirillum undula majris*. Meier used a similar medium to determine the numbers of bacteria in milk and milk products.

Variants: Meier prepared a medium as follows:

(1) Boil 500.0 g. of fat and tendon free beef in 1 liter water.
(2) Filter.
(3) Dissolve 15.0 g. agar and 5.0 g., 20.0 g. or 40.0 g. lactose or glucose in the filtrate.
(4) Neutralize by the addition of KOH. Add KOH until turmeric paper is turned quite weakly brownish red.
(5) Sterilization not specified.

References: Kutscher (1895 p. 615), Meier (1918 p. 436).

2171. Gassner's Basal Fuchsin Infusion Agar

Constituents:

1. Water	750.0 cc.
2. Meat infusion	250.0 cc.
3. Agar	30.0 g.
4. NaCl	5.0 g.
5. Fuchsin (acid) (3.0% soln.)	60.0 cc.

Preparation:

(1) Prepare meat infusion (method not given).
(2) Mix 750.0 cc. of water with 250.0 cc. of (1).
(3) Dissolve 30.0 g. agar and 5.0 g. NaCl in (2).
(4) Adjust to a slight alkalinity using litmus as an indicator.
(5) Dissolve one of the added nutrients in (4).
(6) To each 80.0 cc. of (5) add 5.0 cc. of a 3.0% acid fuchsin solution and 0.0, 0.1, 0.2, 0.5, or 1.0% glucose.

Sterilization: Not specified.

Use: To study nitrogen source requirements for colon-typhoid and dysentery group. The author reported that asparagin was utilized as a nitrogen source by the typhoid and dysentery group as well as by *Bacterium coli*.

Added nutrients: The author added 10.0 g. asparagin or 20.0 g. peptone.

Variants: The author added 0.1, 0.2, 0.5, or 1.0% glucose. See step (6) above.

Reference: Gassner (1917–18, p. 263).

2172. Harde and Hauser's Fish Infusion Agar

Same as medium 1346, but solidified by the addition of 15.0 g. agar.

2173. Pergola's Mussel Infusion Agar

Solidify medium 1347 by the addition of 2.0% agar.

2174. Harvey's Glucose Starch Infusion Agar

Constituents:

1. Water	1500.0 cc.
2. Beef	500.0 g.
3. Agar	23.0 g.
4. Starch	15.0 g.
5. Glucose	15.0 g.
6. Litmus solution	

Preparation:

(1) Macerate 500.0 g. finely minced beef in 1500.0 cc. water in the ice chest over night.
(2) Filter thru doubled gauze.
(3) Bring the filtrate to the boil and add agar 1.5%.
(4) Boil 25 minutes.
(5) Estimate and adjust reaction to 0.5% acid to phenolphthalein.
(6) Clarify and filter.
(7) Add 1.0% starch, 1.0% glucose and color the medium with litmus solution.
(8) Steam 45 minutes shaking 3 times during this period to distribute the starch.
(9) Distribute into test tubes.

Sterilization: Sterilize 15 minutes at 10 pounds.

Use: Differentiation of meningococci and *M. catarrhalis.* Author reported that meningococci acidified with glucose, *M. catarrhalis* did not.

Reference: Harvey (1921–22 p. 111).

2175. Vedder's Starch Infusion Agar

Constituents:

1. Beef infusion (not containing peptone or salt)	1000.0 cc.
2. Starch, corn	10.0 g.
3. Agar	15.0 to 17.5 g.

Preparation:

(1) Exact composition of beef infusion not given except that it should contain no peptone or salt.
(2) Dissolve agar in beef infusion, cook, clarify and filter according to methods of preparing ordinary agar.
(3) Neutralize so that final reaction will be from 0.2 to 0.5% acid to phenolphthalein.
(4) Grind corn starch in mortar with a little of (3) to avoid lumps.
(5) Boil (4) for a few minutes.
(6) Tube.

Sterilization: Sterilize at not more than 15 pounds pressure.

Use: Special culture medium. Author reported that medium will keep gonococci alive for long period of time. May be used to isolate gonococci and for routine purposes. Park, Williams and Krumwiede recommended a similar medium for the cultivation of meningococci.

Variants:

(a) Harvey prepared the medium as follows:
  (1) Macerate 500.0 g. finely minced beef in 1500.0 cc. water in the ice chest over night.
  (2) Filter thru doubled gauze.
  (3) Bring the filtrate to the boiling point and add 1.5% agar.
  (4) Boil 25 minutes.
  (5) Estimate and adjust reaction to 0.5% acid to phenolphthalein.
  (6) Clarify and filter.
  (7) Add 1.0% starch.
  (8) Steam 45 minutes shaking 3 times during this period to distribute the starch.
  (9) Distribute into test tubes.
  (10) Sterilize 15 minutes at 10 pounds pressure.

  He reported that there should be an abundance of water of condensation developed, and the agar must not exceed 1.5%.

(b) Park, Williams and Krumwiede prepared a medium as follows:
  (1) Extract 500.0 g. beef in 1000.0 cc. of water in an ice box over night.
  (2) Boil and strain.
  (3) Dissolve 1.5% agar in (2).
  (4) Adjust the reaction to +0.2 to +0.7 to phenolphthalein (pH between 7.0 and 7.6).
  (5) Cool and clarify with eggs.
  (6) Filter.
  (7) Add 1.0% starch and heat in the Arnold for 45 minutes shaking the medium several times to distribute the starch.
  (8) Tube.
  (9) Autoclave 15 minutes at 10 pounds pressure.

References: Vedder (1914 p. 385), Tanner (1919 p. 60), Giltner (1921 p. 371), Harvey (1921–22 p. 111), Park, Williams and Krumwiede (1924 p. 131).

2176. Kopp's Thyroid Agar

Constituents:

1. Water	1000.0 cc.
2. Thyroid gland, sheep	500.0 g.
3. Agar	10.0 g.
4. Glycerol	30.0 cc.
5. NaCl	5.0 g.

Preparation:

(1) Free sheep thyroid glands from fat, and chop the glands into small pieces.
(2) Extract the glands for 3 hours with an equal weight of sterile water.
(3) Add (2) to moistened linen and press the fluid thru.
(4) Dissolve 3, 4 and 5 in 500.0 cc. water.
(5) Mix equal parts of sterile (4) and sterile (3) melted and cooled to 40°C.

Sterilization: Filter (3) thru a clay filter to sterilize. Method of sterilization of agar not given.

Use: Cultivation of colon-typhoid group.

Reference: Kopp (1895 p. 81).

2177. Wróblewski's Suprarenal Capsule Agar

Constituents:

1. Water	500.0 cc.
2. Suprarenal capsule (ox)	100.0 g.
3. Agar	7.5 g.
4. Glycerol	18.0 g.
5. NaCl	3.0 g.

Preparation:

(1) Remove the suprarenal capsule from an ox immediately after death.
(2) Chop to small bits and rub to a pulp.
(3) To 100.0 g. of (2) add 200.0 cc. of water.
(4) Make (3) alkaline with soda and boil for 2 hours. (Filtration not specified).
(5) Dissolve 7.5 g. agar, 18.0 g. glycerol and 3.0 g. of NaCl in 300.0 cc. of water.
(6) Mix equal quantities of (5) and (4).
(7) Sterilize (method not given).
(8) Filter two or three times to clarify.

Sterilization: Sterilize the filtrate (method not given) and store at 36°C. for several days to test sterility.

Use: General culture medium. Author reported that the medium suported the growth of some organisms better than others.

Reference: Wróblewski (1896 p. 528).

2178. Ficker's Brain Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Brain, beef	500.0 g.
3. Agar (2.5%)	12.5 g.
4. Glycerol	30.0 g.

Preparation:

(1) Pass 500.0 g. fresh brain thru a meat chopping machine.
(2) Add an equal weight (500.0 cc.) of distilled water.
(3) Stir and heat slowly to a boil.
(4) Boil slowly for 15 minutes.
(5) Filter thru a filtering cloth until the filtrate assumes a pulpy character. Press the coagulated mass free from any liquid.
(6) Prepare a 2.5% agar solution in distilled water and filter.
(7) Mix equal parts of (6) and sterile (5).
(8) Add 3.0% glycerol.
(9) Tube sterilized (8).
(10) Cool to 45°C. Mix well and solidify quickly in a slanted position.

Sterilization: Sterilize the filtrate from (5) by steaming for two hours. Sterilization of the agar not specified. Sterilize (8) by heating in steam for 30 minutes.

Use: Cultivation of tubercle bacilli.

Variants:

(a) The author reported that human, sheep, beef, calf and horse brain were used, but all gave the same results.
(b) Abel boiled the brain infusion for 45 minutes instead of 15 minutes as in step (4) above.

References: Ficker (1900 p. 593), Abel (1912 p. 90), Kolle and Wasserman (1912 p. 410), Klimmer (1923 p. 224).

2179. Ficker's Spleen Agar

Same as medium 2178 but substituting beef spleen for brain.

2180. Duval's Turtle Agar

Constituents:

1. Distilled water	500.0 cc.
2. Agar	20.0 g.
3. NaCl	3.0 g.
4. Glycerol	30.0 cc.
5. Muscle, turtle	500.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Clarify and tube in 10.0 cc. lots.
(3) Cut 500.0 g. turtle muscle into 500.0 cc. distilled water.
(4) Keep in the ice chest 48 hours and filter thru gauze to remove the tissue.
(5) Add 5.0 cc. of sterile (4) to each tube of sterile (2) that has been melted and cooled to 42°C.
(6) Agitate thoroly and allow to solidify in the slanted position.

Sterilization: Sterilize (2) in the usual manner, method not given. Sterilize (4) by filterting thru a Berkefeld filter.

Use: Cultivation of *B. leprae*. Author reported that growth was moist and orange yellow in color. Growth reached a maximum in 48 to 64 hours.

Reference: Duval (1911 p. 370).

2181. Loeffler's Malachite Green Nutrose Agar

Constituents:

1. Water	2000.0 cc.
2. Beef	1 lb.
3. Agar	30.0 g.

4. Nutrose (10.0% solution)
5. Malachite Green

Preparation:

(1) Prepare a beef infusion of 1 pound in 2 liters water.
(2) Add 30.0 g. agar.
(3) Add 7.5 cc. N/1 HCl.
(4) Boil 30 minutes.
(5) Add 7.0 cc. N/1 NaOH.
(6) Neutralize to litmus with sodium carbonate.
(7) Add 5.0 cc. N/1 sodium carbonate.
(8) Add nutrose to make 1.0% solution using a 10.0% solution of nutrose for this purpose. (0.5% peptone may be used instead of nutrose.)
(9) Boil.
(10) Distribute in 500.0 cc. flasks.
(11) Let cool slowly in sterilizer following sterilization.
(12) Decant clear supernatant liquid.
(13) To each 100.0 cc. agar add 2.0 to 2.5 cc. of a 2.0% heated malachite green solution, prepared with sterile water.
(14) Pour 15.0 to 20.0 cc. to each Petri dish.
(15) Leave dishes open until agar is cool and hard.

Sterilization: Sterilize (10) several hours on each of 2 successive days in streaming steam.

Use: Isolation of typhoid bacilli from feces.

References: Loeffler (1906 p. 289), Klimmer (1923 p. 212).

2182. Meyer's Tissue Agar

Constituents:

1. Brain infusion agar	1000.0 cc.
2. Glucose	5.0 g.
3. Tissue (guinea pig)	

Preparation:

(1) Prepare brain infusion agar (details of method not given).
(2) Dissolve 5.0 g. glucose in (1) and adjust to reaction +0.5.
(3) Boil, cool and inoculate sterile (2) with edema, abdominal fluid or heart blood of infected animal, properly diluted.
(4) Cut sterile (method not given) guinea pig tissue into small pieces and distribute them over the bottom of a sterile Petri dish.
(5) Pour (3) over tissues, and solidify agar quickly on an iced glass plate.
(6) Place plates in Novy jar, remove oxygen and fill with hydrogen.

Sterilization: Method of sterilization of agar not given.

Use: Cultivation of organism causing symptomatic anthrax or black-leg in swine. The author reported that other tissues could not replace that of the guinea pig.

Reference: Meyer (1915 p. 467).

2183. Williams and Burdick's Egg Veal Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Glycerol (15.0%)	150.0 g.
3. Egg white (10.0% soln.)	300.0 cc.
4. Egg yolk (10.0%)	300.0 cc.
5. Veal	500.0 g.
6. NaCl	5.0 g.
7. Agar	15.0 g.
8. Gentian violet (1.0% alcoholic solution)	1.0 cc.

Preparation:

(1) Prepare 300.0 cc. of a 10.0% filtered aqueous solution of egg white.
(2) Prepare 300.0 cc. of a 10.0% filtered aqueous solution of egg yolk. To produce the proper turbidity add 1.0 cc. of normal NaOH for each 100.0 cc. of emulsion.
(3) Add 500.0 g. chopped lean veal to 1000.0 cc. of 15.0% glycerol solution in water.
(4) Infuse (3) for 24 hours.
(5) Filter (4).
(6) Add 5.0 g. NaCl to (5) and heat to boiling.
(7) Filter and render +1.0% alkaline.
(8) Add 15.0 g. of powdered agar to (7).
(9) Add 1.0 cc. of a 1.0% alcoholic solution of gentian violet to sterile warm (8).
(10) Pour sterile (9) into sterile (1).
(11) Add sterile (10) to sterile (2).
(12) Mix, tube and slant.

Sterilization: Sterilize (1), (2) and (8) separately in the autoclave at 15 pounds pressure for 15 minutes.

Use: Cultivation of *B. tuberculosis*. The authors reported that medium contained sufficient moisture to prevent its drying up under ordinary conditions and was not liquefied by fast growing types.

Reference: Williams and Burdick (1916 p. 413).

2184. Burger's Basal Lead Acetate Agar

Constituents:

1. Water	1000.0 cc.
2. Agar	30.0 g.
3. Lead acetate	1.0 g.
4. Meat extract	4.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Suspend 1.0 g. of one of the added nutrients in physiological salt solution and add 3.0% Na_2CO_3.

(3) Add (2) to melted (1), cooled to 56°C.

Sterilization: Not specified.

Use: To study the production of hydrogen sulphide.

Added nutrients: The author suspended 1.0 g. of one of the following in physiological salt solution:

cystine
taurine
sodium taurocholate

Reference: Burger (1914 p. 202).

2185. Hiss' Extract Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Extract, Liebig's	5.0 g.
3. NaCl	5.0 g.
4. Agar	15.0 g.

Preparation:

(1) Dissolve agar in water by boiling over free flame for 30 to 45 minutes.

(2) Dissolve Liebig's extract and sodium chloride in (1).

(3) Clarify by the coagulation of two egg whites in (2).

(4) Filter thru cotton.

(5) Reaction is 0.75% acid to phenolphthalein.

(6) Tube in 10.0 cc. lots.

Sterilization: Sterilize in the Arnold in the usual manner on three successive days.

Use: Differentiation of typhoid, colon and allied forms. The author reported that typhoid colonies were threaded. Colon colonies formed no threads, surface colonies dark at center, light at periphery. Little difference, if any, if salt be omitted. Other authors used similar media for the cultivation of amoeba, parasitic flagellata and ciliata, cladothrix and protozoa.

Variants:

(a) Hiss omitted the NaCl.

(b) Musgrave and Clegg cultivated amoeba on a medium containing 20.0 g. agar, 0.3 to 0.5 g. NaCl and 0.3 to 0.5 g. beef extract per liter. The original reaction of the medium being 1.5% alkaline before sterilization, and 1.0% alkaline following sterilization. The authors reported that in some cases even smaller amounts of salt and extract were more desirable—especially when amoeba were growing in company with some saprophytic bacteria.

(c) Linde isolated cladothrix (*Cladothrix dichotoma*) on a medium containing 15.0 g. agar and 5.0 g. meat extract per liter. Linde reported that contaminating forms grew but cladothrix soon overgrew them. The best solid medium for cultivation is 10.0 g. agar with 0.5 g. meat extract in 1000.0 cc. water.

(d) Zikes cultivated *Cladothrix dichotoma* and *Cladothrix natans* on Linde's medium, containing 5.0 g. meat extract and 10.0 g. agar per liter.

(e) Malm (Besson) prepared the medium as follows:

(1) Dissolve 5.0 g. Liebig's meat extract (or 20.0 g. Cibils) in 1000.0 cc. water.

(2) Soak 20.0 g. of chopped thread agar in cold water for several hours. Squeeze the water thru a cloth.

(3) Heat (2) in (1) at 100°C. until the agar is dissolved.

(4) Readjust the reaction if necessary.

(5) Allow to cool to 55 or 60°C.

(6) Beat the white of an egg in 100.0 cc. of water and add to (5).

(7) Mix well.

(8) Autoclave at 120°C. for one hour.

(9) Filter thru a moistened Chardin filter using a hot water funnel.

(10) Tube.

(11) Sterilize at 115° for 20 minutes.

(f) Harvey cultivated protozoa on a

medium containing 0.4 g. Lemco meat extract, 0.4 g. NaCl and 25.0 g. agar per liter. The reaction of the medium was 1.0% alkaline to phenolphthalein.

References: Hiss (1902 p. 158), Musgrave and Clegg (1905 p. 335), Walker (1908 p. 490), Linde (1913 p. 373), Zikes (1915 p. 543), Besson (1920 p. 43), Harvey (1921–22 p. 94).

2186. Bacto Starch Agar (Dehydrated)

Constituents:

1. Distilled water	
2. Beef extract, Bacto	3.0 g.
3. Agar, Bacto	12.0 g.
4. Starch, Soluble, Difco	10.0 g.

Preparation:

(1) Dissolve 25.0 g. of Bacto starch agar (dehydrated) in 1000.0 cc. of distilled water by boiling or autoclaving, preferably the latter.

(2) Restore loss if necessary.

(3) Distribute as desired.

Sterilization: Sterilize for 20 minutes at 15 pounds pressure.

Use: Culture medium.

Reference: Digestive Ferments Co. (1925 p. 14).

2187. Hiss' Glucose Extract Agar

Constituents:

1. Distilled water	1000.0 cc.
2. Agar	15.0 g.
3. Extract Liebig's	5.0 g.
4. NaCl	5.0 g.
5. Glucose	10.0 g.

Preparation:

(1) Dissolve the agar in water by boiling over a free flame for 30 to 45 minutes.

(2) Dissolve Liebig's extract and salt in (1).

(3) Filter thru paper.

(4) Dissolve the glucose in (3).

(5) The reaction is usually 0.8% acid to phenolphthalein.

(6) Tube in 10.0 cc. lots.

Sterilization: Sterilize in the usual manner at 100°C. on 3 successive days.

Use: Differentiation between typhoid, colon and other allied bacilli. The author reported that the typhoid bacilli gave threaded irregular colonies. Colon colonies were large and smooth. If medium be clarified with egg whites a more alkaline medium was obtained which allowed better differentiation. One cc. NaOH added to the medium also allowed better differentiation.

Variants:

(a) The medium may be clarified with egg white.

(b) One cubic centimeter of normal NaOH may be added.

(c) The medium may be prepared as follows:

  (1) Dissolve 15.0 g. agar in water by boiling for 30 to 45 minutes over a free flame.

  (2) Dissolve 5.0 g. Liebig's extract in (1).

  (3) Coagulate the whites of two eggs in (2) to clarify.

  (4) Filter thru absorbent cotton.

  (5) Dissolve 10.0 g. glucose in (4).

  (6) Reaction is 0.75% acid to phenolphthalein.

  (7) Tube in 10.0 cc. lots.

  (8) Sterilize in the Arnold in the usual manner on three successive days.

Reference: Hiss (1902 pp. 156, 158).

2188. Tanner's Malachite Green Extract Agar

Constituents:

1. Water	1000.0 cc.
2. Meat extract, Liebig	3.0 g.
3. Agar	30.0 g.
4. Sucrose	10.0 g.
5. Malachite green (2.0% solution)	2.0 to 2.9 cc.

Preparation:

(1) Dissolve 2 in 1.

(2) Acidify with 7.5 cc. normal HCl.

(3) Dissolve 30.0 g. agar in (2) by boiling.

(4) Neutralize by adding 7.0 cc. normal NaOH until neutral to litmus.

(5) Add 5.0 cc. of normal Na_2CO_3.

(6) Heat in an Arnold for several hours.

(7) Add 100.0 cc. of 10.0% sucrose solution to (6).

(8) Store in 100.0 cc. quantities.

(9) Before use redissolve and add 2.0 to 2.9 cc. of a 2.0% malachite green (trade mark Höchst. 120) to each 100.0 cc. of medium. Prepare the

malachite green solution in sterile water. Do not boil.

(10) Pour in Petri dishes.

Sterilization: Not specified.

Use: General culture medium.

Reference: Tanner (1919 p. 52).

2189. Noyes' Starch Extract Agar

Constituents:

1. Water	1000.0 cc.
2. Agar (best)	15.0 g.
3. Extract (Liebig)	5.0 g.
4. Starch	4.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: General culture medium. Primarily used to study soil organisms.

Reference: Noyes (1916 p. 93).

2190. Malenković's Xylose Extract Agar

Constituents:

1. Water	1000.0 cc.
2. Xylan	20.0 g.
3. Meat extract, Liebig's	5.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: Cultivation of *Merulius lacrymans*.

Reference: Malenković (1906 p. 407).

2191. Hiss' Gelatin Extract Agar

Constituents:

1. Water	1000.0 cc.
2. Beef extract, Liebig's	5.0 g.
3. NaCl	5.0 g.
4. Agar	5.0 g.
5. Gelatin	80.0 g.
6. Glucose	10.0 g.

Preparation:

(1) Dissolve agar in 1000.0 cc. of water to which beef extract and NaCl have been added.

(2) When agar is completely melted dissolve the gelatin by a few minutes boiling.

(3) Titrate and adjust to 1.5% normal acid to phenolphthalein.

(4) Add one or two eggs beaten in 25.0 cc. of water and boil for 45 minutes.

(5) Filter thru thin filter paper or absorbent cotton.

(6) Add the glucose after clearing.

(7) Tube.

Sterilization: Method of sterilization not given.

Use: Differentiation of colon-typhoid group. Author reported that on the medium given above *B. typhosus* gave a uniform clouding. Colon bacilli gave only growth where inoculated.

Variants:

(a) Hiss prepared a similar medium using 10.0 g. agar instead of 5.0 g., and 25.0 g. of gelatin instead of 80.0 g. The reaction is adjusted to 2.0% acid instead of 1.5%. This medium is poured in plates when ready for use. On this medium the author reported that deep *B. typhosus* colonies under low power were small and generally spherical with rough irregular outline. By transmitted light they were of a vitreous greenish or yellowish-green color. Showed threaded growth. Deep colon colonies were usually much larger and were spherical or of whetstone form. By transmitted light they were darker more opaque and less refractive than typhoid colonies. By reflected light they were pale yellow. Neither of the organisms spread thru the medium. The typhoid colonies appeared to the naked eye as mere greyish points among the much larger and yellowish colon colonies.

(b) Hiss gave the following method of preparation of a similar medium. He reported that typhoid colonies were small greenish, irregular and fringed with threads. Colon colonies were larger and did not form threads.

(1) Boil 15.0 g. agar in 1000.0 cc. distilled water over a free flame for 30 to 45 minutes to dissolve.

(2) Dissolve 15.0 g. gelatin, 5.0 g. Liebig's extract and 5.0 g. NaCl in (1).

(3) Clarify by the addition and coagulation of the whites of two eggs.

(4) Filter thru absorbent cotton.

(5) Dissolve 10.0 g. glucose in (4).

(6) The reaction is usually about 1.2% acid to phenolphthalein and no acid or alkali is added.

(7) Tube in about 10.0 cc. lots.

(8) Sterilize in the usual manner at 100°C. on 3 successive days.

References: Hiss (1897 pp. 681, 694), (1901 p. 728), (1902 pp. 151, 152, 156), Frost (1903 p. 340), Ball (1919 p. 80), Tanner (1919 pp. 51, 56) Heinemann (1922 p. 34).

2192. Stapp's Egg White Extract Agar

Constituents:

1. Water	500.0 cc.
2. Egg white	
3. Meat extract	1.33 g.
4. NaCl	0.33 g.
5. Glucose	1.66 g.
6. Agar	10.0 g.

Preparation:

(1) Beat fresh egg white until it is a completely uniform liquid.
(2) Mix with water in the ratio of 1:100 by thoro shaking.
(3) Close the flask and heat in the steamer for 20 minutes.
(4) When all the air is removed from the flask place the flask in the autoclave and heat at 138° for 20 minutes.
(5) Remove after cooling and filter.
(6) Mix 200.0 g. of the filtrate, 0.33 g. NaCl, 1.66 glucose, 10.0 g. agar, 1.33 g. meat extract and 300.0 cc. water. (The meat extract may be omitted).
(7) Dissolve by heating in the steamer.
(8) Filter while hot.

Sterilization: Not specified.

Use: Cultivation of *Bac. cobayae*, *Bac. capri*, *Bac. guano*, *Bac. musculi*, *Bac. hollandicus*.

Reference: Stapp (1920 p. 5).

SUBGROUP II-C. SECTION 10

Basal or complete media containing agar with derivatives of soil, ashes, etc., but not containing digests.

A₁. Ashes or derivatives employed.
  Harrison and Barlow's Wood Ash Agar ... 2193
A₂. Soil infusions or extracts employed.
  Fremlin's Soil Infusion Agar ... 2194
  Gowda's Soil Infusion Agar ... 2195
  Conn's Soil Infusion Agar ... 2196
  Löhnis' Mannitol Soil Infusion Agar ... 2197
  Perotti's Dicyandiamide Soil Infusion Agar ... 2198

2193. Harrison and Barlow's Wood Ash Agar

Constituents:

1. Water	200.0 cc.
2. Ashes, wood	5.0 g.
3. Agar	2.0 g.
4. Maltose	2.0 g.

Preparation:

(1) Dissolve 2.0 g. agar and 2.0 g. maltose in 100.0 cc. water by heat.
(2) Add 5.0 g. wood ashes to 100.0 cc. water.
(3) Boil and filter.
(4) Mix 100.0 cc. of (1), 40.0 cc. of (2), and 60.0 cc. of water or mix 100.0 cc. (1) with 60.0 cc. (2) and add 40.0 cc. of water or mix 100.0 cc. (1) with 100.0 cc. of (2) and add no water.

Sterilization: Sterilize in streaming steam or in the autoclave at 10 pounds for 10 to 20 minutes.

Use: Cultivation of *Pseudomonas radicicola* and other bacteria from the nodules of leguminous plants.

Variants:

(a) Harrison and Barlow gave a number of different media, using varying amounts of constituents, as in medium 1377, and then adding 1.2, 1.5 or 2.0% agar to solidify.
(b) Löhnis prepared the medium as follows:
  (1) Infuse 4.0 g. of wood ashes with 1000.0 cc. of water.
  (2) Add 4.0% maltose and 2.0% K_2HPO_4 to (1).
  (3) Solidify with agar.
  (4) Sterilization not specified.
(c) Harvey dissolved 4.0 g. maltose, 5.0 g. wood ashes and 10.0 g. agar in 1000.0 cc. distilled water.
(d) Percival prepared the medium as follows:
  (1) Add 8.0 g. of well burnt wood ashes to 500.0 cc. distilled water and boil for one minute.
  (2) Filter thru two sheets of paper.
  (3) Add 4.0 g. maltose and 4.0 g. of agar to (2).
  (4) Heat until dissolved.
  (5) Filter.
  (6) Tube.
  (7) Sterilize in the usual way, method not given.
  (8) Slant.
(e) Giltner gave the following method of preparation:
  (1) Stir 5.0 g. of wood ashes (elm, beech, maple) into 1000.0 cc. of

distilled water for two or three minutes only.

(2) Filter.
(3) Add 1.0% washed agar.
(4) Heat in steam for 30 minutes.
(5) Add 1.0% commercial sucrose.
(6) Boil for 5 minutes over a free flame.
(7) Strain while hot thru several thicknesses of clean cheese cloth. This may be filtered if desired.
(8) Sterilize by the Tyndall method in the steamer.

References: Harrison and Barlow (1907 p. 269), Löhnis (1913 p. 113), Harvey (1921–22 p. 111), Percival (1920 p. 204), Giltner (1921 p. 377).

2194. Fremlin's Soil Infusion Agar

Constituents:

1. Soil infusion	1000.0 cc.
2. Agar	

Preparation:

(1) Solidify soil infusion with agar.

Sterilization: Not specified.

Use: Cultivation of nitroso bacteria. Francois-Perey cultivated soil protozoa on a similarly prepared medium

Variants:

(a) Löhnis prepared the medium as follows:
  (1) Heat 1000.0 cc. of good rich garden soil with a liter of tap water for 30 minutes in the autoclave under pressure of 1 atmosphere or boil with 2 liters of water over a free flame.
  (2) Pour off the turbid liquid.
  (3) Mix talc with the liquid.
  (4) Filter thru a double filter paper.
  (5) Make up the volume to 800.0 cc. if necessary.
  (6) Solidify with agar.
  (7) Sterilize (method not given).
(b) Francois-Perey prepared the medium as follows:
  (1) Boil 1000.0 g. soil with 2000.0 cc. of water.
  (2) Filter and dissolve 2.0% agar in the filtrate.

References: Fremlin (1903 p. 373), Löhnis (1913 p. 101), Giltner (1921 p. 370), Francois-Perey (1925 p. 315), taken from (1925 p. 93).

2195. Gowda's Soil Infusion Agar

Constituents:

1. Water	2000.0 cc.
2. Soil	1000.0 g.
3. K_2HPO_4	1.5 g.
4. $(NH_4)_2SO_4$	1.5 g.
5. $MgSO_4$	0.75 g.
6. Fe_2SO_4	0.02 g.
7. NaCl	3.0 g.
8. Na_2CO_3	1.5 g.
9. Agar	

Preparation:

(1) Digest 1000.0 g. soil in the autoclave for 2 hours with 2 liters of water.
(2) Soak agar in water for 8 to 10 days.
(3) Dissolve 3 in 100.0 g. conductivity water.
(4) Dissolve 4, 5 and 6 in 100.0 g. conductivity water.
(5) Dissolve 7 and 8 in 100.0 g. conductivity water.
(6) Add 1.0 cc. each of sterile (3), (4) and (5) to each agar plate. The details of preparation of the plates are not given, or the amount of soil extract that is to be added to the plate, or if the washed agar is to be dissolved in the soil extract is not stated.

Sterilization: Pass the liquid from (1) thru a Chamberland filter, then sterilize on 3 successive days in the autoclave under 15 pounds pressure for four hours. Sterilize (3), (4) and (5) separately, method not given.

Use: To study nitrification. Gowda reported that the ammonia was oxidized to nitrites.

Reference: Gowda (1924 p. 255).

2196. Conn's Soil Infusion Agar

Constituents:

1. Water	900.0 cc.
2. Soil infusion	100.0 cc.
3. Glucose	1.0 g.
4. Agar	15.0 g.

Preparation:

(1) Preparation of soil infusion not given.
(2) Dissolve 3 and 4 in 1.
(3) Mix (1) and (2).

Sterilization: Not specified.

Use: Cultivation of soil bacteria.

Variants: Harvey prepared a similar medium as follows:

(1) Dissolve 15.0 g. agar in 900.0 cc. of water.
(2) Boil 2000.0 cc. water and 1000.0 g. garden soil for 2 hours.
(3) Pour off the turbid fluid.
(4) Mix talc with (3).
(5) Filter until clear thru thick filter paper.
(6) Mix 100.0 cc. of (5) and 900.0 cc. of (1).
(7) The reaction should be 0.5% acid to phenolphthalein.
(8) Add 1.0 g. glucose to (7).
(9) Tube.
(10) Sterilization not specified.

References: Tanner (1919 p. 49), Harvey (1921–22 p. 105).

2197. Löhnis' Mannitol Soil Infusion Agar

Constituents:

1. Water	1000.0 cc.
2. Soil	1000.0 g.
3. Mannitol (1.0%)	10.0 g.
4. K_2HPO_4 (0.05%)	0.4 g.
5. Agar (1.5%)	12.0 g.

Preparation:

(1) Heat 1000.0 g. of good rich garden soil with a liter of tap water for 30 minutes in the autoclave under pressure of 1 atmosphere or boil with 2 liters of water over a free flame.
(2) Pour off the turbid liquid.
(3) Mix talc with the liquid.
(4) Filter thru a double filter paper.
(5) Make up the volume to 800.0 cc. if necessary.
(6) Dissolve 3, 4 and 5 in (5).
(7) Tube.

Sterilization: Sterilize in the autoclave or steamer.

Use: Isolation of nodule bacteria.

Reference: Löhnis (1913 p. 112).

2198. Perotti's Dicyandiamide Soil Infusion Agar

Same as medium 1392 but solidified by the addition of 1.5% agar.

GROUP III. MEDIA SOLIDIFIED BY ADDITION OF GELATIN

SUBGROUPS OF LIQUEFIABLE GELATIN MEDIA

A_1. Not containing organic materials other than gelatin
Subgroup III-A (Med. 2199–2204)
A_2. Containing organic material in addition to gelatin
Subgroup III-B (Med. 2205–2371)

SUBGROUP III-A

Gelatin Media, with Additional Constituents Inorganic

SUBGROUP III-A

Basal or complete media containing gelatin. Additional constituents, if any, inorganic.

A_1. Containing gelatin and water only.
Natsuschita's Gelatin 2199
Taylor's Salt Free Gelatin 2200
Matzuschita's Basal Gelatin 2201
A_2. Containing added salts.
Stutzer and Hartleb's Phosphate Gelatin 2202
Stoklasa's "Kollagen" 2203
Söhngen's Magnesium Phosphate Gelatin (Vierling) 2204

2199. Matzuschita's Gelatin

Constituents:
1. Water .... 1000.0 cc.
2. Gelatin .... 100.0 g.

Preparation:
(1) Thoroly shake 100.0 g. gelatin in one liter of water.
(2) Heat until the gelatin is melted.
(3) Neutralize (indicator not specified).
(4) Boil in the steamer.
(5) Filter.

Sterilization: Sterilize in the steamer on from 2 to 5 successive days for 15 to 30 minutes. Incubate for 2 days at 37°C. to test sterility

Use: Used by Matzuschita for the cultivation of spore forming bacilli, *Clostridium butyricum, Bacillus oedematis maligni, Bacillus anthracis symptomatici, Bacillus sporogenes, Bacillus botulinus.* Other investigators used similar media for a variety of purposes.

Variants:
(a) Kufferath used a 10.0, 20.0, 30.0, 40.0 or 70.0 gelatin solution.
(b) Meyer used a 10.0% gelatin solution, adjusted to 0.4% to attempt to cultivate the causative agent of symptomatica anthrax, or blackleg in swine.
(c) Conn (1916) cultivated spore forming bacteria on a 12.0% gelatin in tap water. The medium was clarified with white of egg.
(d) Conn (1917) cultivated soil microorganisms in a medium prepared by dissolving 200.0 g. of Gold Label gelatin in 1000.0 cc. tap water, adjusting to 0.5% normal acid to phenolphthalein (20.0 to 30.0 cc. of normal NaOH). He reported that 120.0 g. of Bacto or United States Glue Co. gelatin might be used instead of Gold Label. Only 10.0 cc. of normal NaOH were required to give the desired reaction. It was necessary to clarify the medium, prepared with United States Glue Co. gelatin, by the addition of white of an egg, but clarification was not necessary when using Bacto gelatin.
(e) Committee S. A. B. gave the following method of preparation:
  (1) Dissolve 120.0 g. Gold Label, 100.0 g. Bacto, or 100.0 g. U. S. Glue Co. gelatin in 1000.0 cc. distilled water.
  (2) Clarify with white of egg.
  (3) Adjust the pH between 6.6 and 7.4.
  (4) Sterilize.
(f) Waksman studied the metabolism of actinomycetes in a 15.0% gelatin solution.
(g) Weiss dissolved 15.0% gelatin in water and used the medium to determine the bacterial count of water.
(h) Harvey cultivated bacteria found in soil on a medium prepared from one

part gelatin to five parts of tap water. The reaction was adjusted to 0.5% acid to phenolphthalein.

References: Matzuschita (1902 p. 287), Kufferath (1914 p. 559), Meyer (1915 p. 467), Conn (1916 p. 188), (1917 p. 42), Committee S. A. B. (1918 p. 116), Waksman (1920 p. 22), Weiss (1920 p. 25), Harvey (1921–22 p. 105), Committee S. A. B. (1923 p. 10).

2200. Taylor's Salt Free Gelatin

Constituents:

1. Distilled water	1000.0 cc.
2. Gelatin	100.0 g.

Preparation:

(1) Soak gelatin in weak alkali and after it has become swollen, wash repeatedly by decantation.

(2) Dissolve gelatin in water by heat.

(3) Precipitate by the least effective quantity of alcohol.

(4) Wash with alcohol and ether and dry.

(5) Dissolve 100.0 g. of (4) in 1000.0 cc. of twice distilled water (conductivity at 16°C. to be 1.1×10^{-6}).

(6) Submit the solution to electrolysis for five days, the conductivity reaching a constant (8×10^{-6}). During electrolysis, dialysis is taking place at all times by means of a special apparatus. A 110 volt circuit was used.

Sterilization: Not specified.

Use: Salt free medium. The author reported that the medium did not support the growth of ordinary saprophytic or pathogenic bacteria.

Reference: Taylor (1905 p. 114).

2201. Matzuschita's Basal Gelatin

Constituents:

1. Gelatin	10.0%

Preparation: (1) Solidify one of the added nutrients by the addition of 10.0% gelatin.

Sterilization: Method not specified.

Use: Cultivation of intestinal bacteria.

Added nutrients: The author used one of the following:

Beer wort
Urine
Straw Infusion

Reference: Matzuschita (1901–02 p. 214).

2202. Stutzer and Hartleb's Phosphate Gelatin

Constituents:

1. Water	1000.0 cc.
2. Gelatin	100.0 g.
3. Potassium phosphate	1.0 g.

Preparation:

(1) Dissolve 100.0 g. of gelatin and 1.0 g. of potassium phosphate in water.

(2) Make up the volume to 1 liter.

(3) Add soda to obtain a neutral reaction.

Sterilization: Method not specified.

Use: Cultivation of *Zoogloea ramigera*, a nitrate fungus.

Reference: Stutzer and Hartleb (1897 p. 236).

2203. Stoklasa's "Kollagen"

Constituents:

1. Water	1000.0 cc.
2. "Kollagen" (c.p. with 17.02% N "Ossein")	
3. K_2SO_4	1.0 g.
4. $MgCl_2$	0.5 g.
5. Iron sulphate	0.1 g.
6. $CaHPO_4$	0.0 or 1.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Sterilize in the autoclave.

Use: To study NH_3 formation by *Bacillus mycoides*, *Bacillus subtilis*, *Bacillus mesentericus vulgatus*.

Reference: Stoklasa (1911 p. 476).

2204. Söhngen's Magnesium Phosphate Gelatin (Vierling)

Constituents:

1. Water	1000.0 cc.
2. Gelatin	100.0 g.
3. $MgSO_4$	1.0 g.
4. K_2HPO_4	1.0 g
5. $CaCl_2$	trace

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Pour sterile (1) into plates.

Sterilization: Not specified.

Use: Cultivation of Mycobacteria. The author reported that the plates were slightly turbid due to a precipitate of $Mg_3(PO_4)_2$. After 8 days mycobacteria caused a clearing of the medium around the colony.

Reference: Vierling (1920 p. 202).

SUBGROUP III-B

Gelatin Media, with Additional Constituents Organic

Key to the Sections of Subgroup III-B

A_1. Chemical composition of all constituents known. Section 1 (Med. 2205–2234)
A_2. Chemical composition of one or more constituents not definitely known.
  B_1. Containing digests
    Section 2 (Med. 2235–2323)
  B_2. Not containing digests
    Section 3 (Med. 2324–2371)

SUBGROUP III-B. SECTION 1

Basal or complete media containing gelatin with additional organic materials of known chemical composition.

A_1. No additional organic nitrogen used.
B_1. Inorganic nitrogen salts not added.
C_1. Containing carbohydrates.
Beijerinck's Starch Gelatin........ 2205
Waksman's Starch Gelatin......... 2206
Dombrowski's Glucose Gelatin..... 2207
Fraenkel's Glucose Glycerol Gelatin. 2208
Beijerinck's Acid Starch Gelatin.. 2209
Bobilioff-Preisser's Basal Glucose Gelatin........................ 2210
C_2. Not containing carbohydrates; other organic carbon used.
Söhngen's Petroleum Gelatin....... 2211
Weisser's Glycerol Gelatin......... 2212
Proskauer and Beck's Glycerol Gelatin (Klimmer).................. 2213
Fermi's Phenol Gelatin........... 2214
B_2. Inorganic nitrogen salts added.
Söhngen's Ammonium Chloride Malate Gelatin.................. 2215
Woltje's Basal Sucrose Gelatin (Zikes)......................... 2216
Mortensen's Sucrose Ammonium Sulphate Gelatin................. 2217
Higgins' Lactose Ammonium Succinate Gelatin.................. 2218
v. Tubeuf's Sucrose Ammonium Citrate Gelatin (Malenkovic)...... 2219
Cohn's Ammonium Tartrate Gelatin (Klimmer)..................... 2220
Kossowicy's Ammonium Phosphate Sucrose Gelatin (Will)........... 2221
v. Tubeuf's Citric Acid Ammonium Nitrate Gelatin................. 2222
Munter's Basal Glucose Salt Gelatin............................. 2223
Mortensen's Basal Nitrate Gelatin.. 2224
A_2. Additional organic nitrogen employed.
B_1. Containing carbohydrates.
C_1. Carbohydrates, only, added.
Beijerinck's Starch Asparagin Gelatin............................ 2225
C_2. Carbohydrates and other organic carbon added.
Grimbert's Starch Asparagin Gelatin (Charrin and Dissard)........ 2226
Kappen's Cyanamide Asparagin Gelatin......................... 2227
Maassen's Glucose Asparagin Gelatin (Klimmer).................. 2228
B_2. Not containing carbohydrates.
C_1. Containing organic acids.
van Delden's Lactate Asparagin Gelatin......................... 2229
Frankel's Lactate Asparagin Gelatin (Klimmer).................. 2231
Uschinsky's Glycerol Asparaginate Gelatin (Klimmer)............... 2232
C_2. Not containing organic acids.
Goslings' Asparagin Gelatin....... 2233
Sullivan's Nitrate Asparagin Gelatin............................ 2234

2205. Beijerinck's Starch Gelatin

Constituents:

1. Water	1000.0 cc.
2. Gelatin	100.0 g.
3. KH_2PO_4	0.5 g.
4. Starch (soluble)	1.0 g.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Pour into plates.

Sterilization: Not specified.

Use: Detection of quinone production. The author seeded the medium by pouring a culture of *S. chromogena* in medium 250 over the plate. After a few days the gelatin was melted and the quinone was extracted with benzol.

Reference: Beijerinck (1900 p. 10).

2206. Waksman's Starch Gelatin

Constituents:

1. Distilled water	1000.0 cc.
2. Gelatin	150.0 g.
3. Starch	10.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Tube.

Sterilization: Method not given.

Use: To study metabolism of actinomycetes.

Reference: Waksman (1920 p. 22).

2207. Dombrowski's Glucose Gelatin

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4$ (0.1%)	1.0 g.
3. K_2HPO_4 (0.2%)	2.0 g.
4. Glucose (5.0%)	50.0 g.
5. Gelatin (10.0%)	100.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of milk yeasts.

Reference: Dombrowski (1910 p. 380).

2208. Fraenkel's Glucose Glycerol Gelatin

Constituents:

1. Water	1000.0 cc.
2. Gelatin	150.0 g.
3. Glycerol	2.5 g.
4. Dextrose	2.5 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Sterilize in the usual manner (method not specified).

Use: Cultivation of nitrate fungi.

Reference: Fraenkel (1898 p. 10).

2209. Beijerinck's Starch Gelatin

Constituents:

1. Water	1000.0 cc.
2. Gelatin	100.0 g.
3. Lactic acid	
4. Starch	

Preparation:

(1) Dissolve 10.0% commercial gelatin in water.

(2) Acidify slightly with lactic acid (a few drops).

(3) Add a little starch (amount not given).

(4) Pour into Petri dishes.

Sterilization: Not specified.

Use: To test for quinone production by *Streptothrix chromogena*. The author reported that the gelatin turned brown due to the oxidation of the formed quinone. The acid hindered this oxidation and stored the quinone. When KI dissolved in dilute HCl, was added the gelatin was colored highly blue as far as the quinone had diffused from the colony.

Reference: Beijerinck (1900 p. 9).

2210. Bobilioff-Preisser's Basal Glucose Gelatin

Constituents:

1. Distilled water	1000.0 cc.
2. Hayduck's Mineral Solution	20.0 cc.
3. Glucose	50.0 g.
4. Gelatin (10.0%)	100.0 g.

Preparation:

(1) Composition of Hayduck's Mineral Solution not given.

(2) Add 20.0 cc. of (1) to 1000.0 cc. of distilled water.

(3) Dissolve 3, 4 and one of the added nutrients in (2).

(4) Distribute in 10.0 cc. lots in small Erlenmeyer flasks.

Sterilization: Sterilize in the steamer.

Use: Cultivation of fungi imperfecti, oöspora. The author reported that the growth was inhibited using inorganic nitrogen source. The gelatin was liquefied.

Added nutrients: The author added one of the following materials:

Asparagin	2.5 g.
Peptone	2.1 g.
$(NH_4)_2SO_4$	2.5 g.

Reference: Bobilioff-Preisser (1916 p. 395).

2211. Söhngen's Petroleum Gelatin

Same as medium 1460 but solidified by the addition of 10.0% gelatin instead of 2.0% agar.

2212. Weisser's Glycerol Gelatin

Constituents:

1. Water	1000.0 cc.
2. Gelatin (10.0%)	100.0 g.
3. Glycerol (2.0%)	20.0 g.

Preparation:

(1) Dissolve 2 and 3 in 1.

(2) Adjust the reaction to a slight alkalinity.

Sterilization: Not specified.

Use: Cultivation of Neapler cholera bacteria (Emmerich).

Reference: Weisser (1886 p. 326).

2213. Proskauer and Beck's Glycerol Gelatin (Klimmer)

Constituents:

1. Water	1000.0 cc.
2. Glycerol	15.0 g.
3. $(NH_4)_2CO_3$	3.5 g.
4. $MgSO_4$ (Crystalline)	2.5 g.
5. KH_2PO_4	1.3 g.
6. Gelatin	100.0 to 150.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli.

Reference: Klimmer (1923 p. 172).

2214. Fermi's Phenol Gelatin

Constituents:

1. Distilled water	1000.0 cc.
2. Gelatin (10.0%)	100.0 g.
3. Phenol (0.5%)	5.0 g.
4. Na_2CO_3 (1.0 or 2.0%)	

Preparation:

(1) Dissolve the gelatin in 1 at 80 to 90°C.
(2) Add 3 and 4 to (1).
(3) Adjust the reaction as desired, by the addition of soda, mineral or organic acids.
(4) Distribute in tubes.

Sterilization: Not specified.

Use: Prove proteolytic enzyme. The author reported that if a proteolytic enzyme was present the gelatin was liquefied.

Variants: The author used 1.0, 2.0 or 5.0% gelatin instead of 10.0%.

Reference: Fermi (1906 p. 177).

2215. Söhngen's Ammonium Chloride Malate Gelatin

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4 (0.05%)	0.5 g.
3. NH_4Cl (0.05%)	0.5 g.
4. Calcium malate (0.5%)	5.0 g.
5. Gelatin (10.0%)	100.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of urea splitting organisms.

Reference: Söhngen (1909 p. 94).

2216. Wöltje's Basal Sucrose Gelatin (Zikes)

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	5.0 g.
3. $MgSO_4$	2.5 g.
4. Sucrose	75.0 g.
5. Gelatin	140.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Dissolve one of the added nutrients in (1).

Sterilization: Not specified.

Use: To study perithezien formation by *Aspergillus oryzae.*

Added nutrients: The author added 10.0 g. of peptone, 10.0 g. asparagin, 10.0 g. $(NH_4)_2SO_4$ to the basal medium.

Variants: Zikes used 5.0 g. K_2SO_4 instead of K_2HPO_4, used 140.0 g. gelatin and used 10.0 g. asparagin as an added nutrient.

References: Zikes (1922 p. 342).

2217. Mortensen's Sucrose Ammonium Sulphate Gelatin

Same as medium 207 but solidified by the addition of gelatin.

2218. Higgins' Lactose Ammonium Succinate Gelatin

Constituents:

1. Water	1000.0 cc.
2. Glucose	2.5 g.
3. Lactose	2.5 g.
4. Ammonium succinate	2.5 g.
5. $(NH_4)_2SO_4$	1.0 g.
6. KCl	1.5 g.
7. KNO_3	2.5 g.
8. Ammonium phosphate	2.5 g.
9. Gelatin (7.0%)	70.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.
(2) Neutralize to phenolphthalein.

Sterilization: Method not given.

Use: Cultivation of organisms causing cholera, Pictoy cattle disease and others.

Reference: Higgins (1898 p. 668).

2219. v. Tubeuf's Sucrose Ammonium Citrate Gelatin (Malenkovic)

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	5.0 g.

3. $MgSO_4$	1.0 g.
4. Ammonium citrate	2.0 g.
5. Gelatin	50.0 g.
6. Cane sugar	50.0 to 100.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of *Merulius lacrymans.*

Reference: Malenkovic (1906 p. 407).

2220. Cohn's Ammonium Tartrate Gelatin (Klimmer)

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	5.0 g.
3. $MgSO_4$ (crystalline)	5.0 g.
4. Ammonium tartrate	10.0 g.
5. Gelatin	100.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Solidify by dissolving 100.0 to 150.0 g. gelatin in (1).

Sterilization: Not specified.

Use: General synthetic culture medium.

Reference: Klimmer (1923 p. 172).

2221. Kossowicy's Ammonium Phosphate Sucrose Gelatin (Will)

Constituents:

1. Water	1000.0 cc.
2. Sucrose (5.0%)	50.0 g.
3. KCl (0.4%)	4.0 g.
4. $(NH_4)_2HPO_4$ (0.4%)	4.0 g.
5. $MgSO_4$ (0.4%)	4.0 g.
6. $CaHPO_4$ (0.04%)	0.4 g.
7. Gelatin (10.0%)	10.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Cultivation of non-spore forming saccharomyces.

Reference: Will (1908 p. 387).

2222. v. Tubeuf's Citric Acid Ammonium Nitrate Gelatin

Constituents:

1. Water	100.0 cc.
2. NH_4NO_3	10.0 g.
3. Potassium phosphate	5.0 g.
4. $MgSO_4$	1.0 g.
5. Citric acid	2.0 g.
6. Gelatin	50.0 g.
7. Glucose	10.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Method not given.

Use: Cultivation of *Merulius lacrymans* causing dry rot. The author reported that a luxuriant white flaky growth was obtained.

Variants: The author used 50.0 or 100.0 g. glucose or 50.0 or 100.0 g. sucrose instead of 10.0 g. glucose.

Reference: v. Tubeuf (1902 p. 130).

2223. Munter's Basal Glucose Salt Gelatin

Same as medium 1442 but solidified by 15.0% gelatin instead of agar.

2224. Mortensen's Basal Nitrate Gelatin

Same as medium 329 but solidified by the addition of gelatin.

2225. Beijerinck's Starch Asparagin Gelatin

Constituents:

1. Water	1000.0 cc.
2. Starch (soluble)	5.0 g.
3. Asparagin	2.5 g.
4. Potassium phosphate	0.5 g.
5. Gelatin	100.0 g.

Preparation: Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Method not given.

Use: Cultivation of *Saccharomyces ellipsoideus.*

Reference: Beijerinck (1895 p. 336).

2226. Grimbert's Starch Asparagin Gelatin (Charrin and Dissard)

Constituents:

1. Distilled water	1000.0 cc.
2. Maltose	1.0 g.
3. Starch (soluble)	2.0 g.
4. Asparagin	2.0 g.
5. Potassium phosphate (neutral)	2.0 g.
6. K_2SO_4	2.0 g.
7. $MgSO_4$	2.0 g.
8. Ammonium bimalate	2.0 g.
9. $MgCO_3$	1.0 g.
10. Gelatin	150.0 g.
11. KI	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

(2) Melt 150.0 g. gelatin in (1) by heating on a salt water bath.

(3) Carefully add KOH until 10.0 cc. of the medium will be neutralized to phenolphthalein by 5.0 cc. of lime solution. (This is an equivalent of 1.0 g. H_2SO_4 per liter.)
(4) Heat in the autoclave for 15 minutes at 110°.
(5) Filter.
(6) Tube in 9.0 cc. lots.
(7) Just before use add 1.0 cc. of a 10.0% KI solution to each tube (liquefied).

Sterilization: Final sterilization not specified.

Use: Isolation of typhoid bacilli. Charrin and Dissard cultivated *Bacillus pyocyaneus* on this medium.

Variants: Remy omitted the KI.

References: Charrin and Dissard (1896 p. 816), Remy (1900 p. 559), Thoinot and Masselin (1902 p. 336).

2227. Kappen's Cyanamide Asparagin Gelatin

Same as medium 473 but solidified by the addition of 10.0% gelatin.

2228. Maassen's Glucose Asparagin Gelatin (Klimmer)

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4$ (crystalline)	0.4 g.
3. Asparagin	10.1 g.
4. $CaCl_2$	0.01 g.
5. Na_2HPO_4	2.0 g.
6. Na_2CO_3 (crystalline)	2.5 g.
7. Glucose	5.0 to 10.0 g.
8. Malic acid	7.0 g.
9. Gelatin	100.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Not specified.

Use: General synthetic culture medium.

Reference: Klimmer (1923 p. 172).

2229. van Delden's Lactate Asparagin Gelatin

Constituents:

1. Water	1000.0 cc.
2. Gelatin	100.0 g.
3. Sodium lactate	5.0 g.
4. Asparagin	1.0 g.
5. $MgSO_4$	1.0 g.
6. K_2HPO_4	0.5 g.
7. Mohr's salt 8. $(FeSO_4)(NH_4)_2SO_4$	trace

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: Isolation of *Microspira desulfuricans* and *Microspira aestuarii.*

Variants: The author added 30.0 g. NaCl for the isolation of *Microspira aestuarii.*

Reference: van Delden (1903–04 p. 88).

2231. Fränkel's Lactate Asparagin Gelatin (Klimmer)

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	2.0 g.
3. NaCl	5.0 g.
4. Ammonium lactate	6.0 g.
5. Asparagin	4.0 g.
6. Gelatin	100.0 g.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Make distinctly alkaline (indicator not specified).

Sterilization: Not specified.

Use: General synthetic culture medium.

Reference: Klimmer (1923 p. 172).

2232. Uschinsky's Glycerol Asparaginate Gelatin (Klimmer)

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	2.0 to 2.5 g.
3. $MgSO_4$ (crystalline)	0.2 to 0.4 g.
4. NaCl	5.0 to 7.0 g.
5. Ammonium lactate	6.0 to 7.0 g.
6. $CaCl_2$	0.1
7. Sodium asparaginate	3.5 g.
8. Glycerol	30.0 to 40.0 g.
9. Gelatin	100.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.

Sterilization: Not specified.

Use: General synthetic culture medium.

Reference: Klimmer (1923 p. 172).

2233. Goslings' Asparagin Gelatin

Constituents:

1. Water	100.0 cc.
2. Gelatin	10.0 g.
3. Asparagin	1.0 g.
4. Na_2SO_4	1.0 g.
5. NaCl	0.5 g.
6. $PbCO_3$	

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of organism causing water rust in flax (*Plectridium pectinovorum*). Author reported that if H_2S be produced the medium was turned brown.

Reference: Goslings (1904 p. 392).

2234. Sullivan's Nitrate Asparagin Gelatin

Constituents:

1. Water	1000.0 cc.
2. Asparagin	10.0 g.
3. $MgSO_4$	0.2 g.
4. $CaCl_2$	trace
5. K_2HPO_4	1.0 g.
6. NaCl	1.0 g.
7. KNO_3	0.2 g.
8. Gelatin	100.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.

Sterilization: Method not given.

Use: Used to determine liquefaction of gelatin by *B. prodigiosus* and *B. pyocyaneus*, and other organisms.

Reference: Sullivan (1905–06 p. 119).

SUBGROUP III-B. SECTION 2

Basal or complete media containing gelatin and a digest.

A_1.* Peptone (or other commercial digest) employed.

B_1. Additional constituents, if any, inorganic.

C_1. Basal media employed with the addition of other materials.
  Will's Basal Salt Peptone Gelatin.. 2235
  Matzuschita's Basal Peptone Gelatin.......... 2236
  Banning's Basal Peptone Gelatin.. 2237
  Harvey's Basal Neutral Red Peptone Gelatin.......... 2238

C_2. Complete media.
  Weiss' Nährstoff Heyden Gelatin... 2239
  Jacobi's Peptone Gelatin.......... 2240
  Metchnikoff's Peptone Gelatin (Thoinot and Masselin).......... 2241
  Mortensen's Peptone Gelatin....... 2242
  Rossi's Salt Peptone Gelatin...... 2243
  Metchnikoff's Nitrate Peptone Gelatin (Bran).......... 2244
  Lichtenstein's Cenovis Gelatin..... 2245

B_2. One or more of the additional constituents organic.

C_1. All additional organic constituents of known chemical composition.

D_1. No additional nitrogen supplied.

E_1. Only one type of organic carbon added.

F_1. Carbohydrates employed.

G_1. Monosaccharides used.
  Utz's Glucose Peptone Gelatin..... 2246
  Beijerinck's Glucose Peptone Gelatin.......... 2247
  Pfeiler and Lentz's Ringer Solution Peptone Gelatin.......... 2248
  Vierling's Glucose Peptone Gelatin. 2249
  Sear's Glucose Peptone Gelatin.... 2250

G_2. Disaccharides used.
  Utz's Lactose Peptone Gelatin..... 2251
  Molisch's Sucrose Peptone Gelatin (Smith).......... 2252
  Trommsdorff's Sucrose Peptone Gelatin.......... 2253

G_3. Polysaccharides used.
  Molisch's Dextrin Peptone Gelatin.. 2254

F_2. Alcohols or organic acids employed.
  Matzuschita's Glycerol Peptone Gelatin.......... 2255
  Molisch's Glycerol Peptone Gelatin. 2256
  Freudenreich and Jensen's Lactate Peptone Gelatin (Boekhout and Ott de Vries).......... 2257

E_2. More than one type of organic carbon added.
  Matzuschita's Glucose Glycerol Peptone Gelatin.......... 2258
  Dombrowski's Glucose Peptone Gelatin.......... 2259

D_2. Additional nitrogen supplied.
  Beijerinck's Asparagin Peptone Gelatin.......... 2260
  Remy's Phenolated Asparagin Peptone Gelatin.......... 2261
  Scholz and Krause's Urea Peptone Gelatin.......... 2262

C_2. One or more of the additional organic constituents of unknown chemical composition.

D_1. Containing unknown constituents of plant origin only.
  Beijerinck's Peptone Malt Gelatin.. 2263
  d'Herelle's Peptone Potato Gelatin. 2264
  Elsner's Peptone Potato Gelatin (Heinemann).......... 2265
  de Rossi's v. Faba Peptone Gelatin. 2266
  Molisch's Pea Gelatin (Löhnis).... 2267
  Molisch's Peat Gelatin.......... 2268

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* See page 720 for A_2.

D_2. Containing unknown constituents of animal origin.

E_1. Animal derivatives (exclusive of extracts and infusions) employed.

F_1. Cells, tissues or their derivatives employed.

Deycke's Peptone Alkali Albumin Gelatin 2269
Kotlar's Pancreas Gelatin 2270
Harde's Tissue Gelatin 2271

F_2. Body fluids employed.

Pergola's Nitrate Alkaline Blood Gelatin 2272

F_3. Secretions or excretions employed.

G_1. Milk or its derivatives added.

Matzuschita's Peptone Milk Gelatin 2273
Raskin's Whey Peptone Gelatin 2274
Appel's Peptone Whey Gelatin 2275
Meier's Whey Peptone Gelatin 2276
Wigger's Whey Gelatin 2277

G_2. Milk or its derivatives not employed.

Piorkoweki's Peptone Urine Gelatine 2278
Heller's Urine Peptone Gelatin 2279

E_2. Animal extracts or infusions employed.

F_1. Infusions specified.

G_1. Additional constituents, if any, inorganic.

Schultz's Peptone Infusion Gelatin 2280
Smith's Peptone Infusion Gelatin 2281
Loeffler's Malachite Green Infusion Gelatin 2282
Roux and Rochaix's Peptone Infusion Gelatin 2283
Müller's Indicator Infusion Gelatin 2284

G_2. Containing additional organic constituents.

Jackson and Muer's Liver Infusion Gelatin 2285
Fuhrmann's Glucose Infusion Gelatin 2286
Choquet's Glycerol Phosphate Infusion Gelatin (Besson) 2287
Teague and Clurman's Congo-Red Brilliant Green Glucose Gelatin 2288
Kowalki's Glycerol Lung Infusion Gelatin 2289
Uffelmann's Methyl Violet Infusion Gelatin 2290
Vincent's Glucose Glycerol Gelatin 2291
Huntoon's Hormone Gelatin 2292

F_2.* Extracts specified.

G_1. Additional constituents, if any, inorganic.

H_1. Basal media employed with the addition of other materials.

Rosenberg's Basal Extract Gelatin 2293
Bürger's Basal Fuchsin Sulphite Extract Gelatin 2294
Tausz and Peter's Basal Ragit Gelatin 2295
Buchan's Basal Litmus Extract Gelatin 2296

H_2. Complete media.

Bacto Nutrient Gelatin (Dehydrated) 2297
Heinemann's Peptone Extract Gelatin 2298
Frost's Peptone Extract Gelatin 2299
Kohn's Peptone Extract Gelatin 2300
Buchan's Neutral Red Peptone Extract Gelatin 2301
von Varon's Nitrate Extract Gelatin 2302

G_2. Containing additional organic constituents.

Henneberg's Glucose Extract Gelatin 2303
Gottheil's Glucose Extract Gelatin 2304
Buchner's Sucrose Extract Gelatin 2305
Percival's Urea Extract Gelatin 2306

F_3. Whether extracts or infusions employed not definitely stated.

G_1. Additional constituents, if any, inorganic. (Exclusive of indicators.)

Zipfel's Basal Nutrient Gelatin 2307
Wurtz's Nutrient Gelatin 2308
Heller's Indicator Nutrient Gelatin 2309

G_2. Containing additional organic materials.

H_1. Chemical composition of additional constituents known.

Matzuschita's Glucose Gelatin 2310
Ramond's Rubine Acid Lactose Gelatin 2311
Wurtz's Litmus Lactose Gelatin 2312
Wurtz's Glycerol Gelatin 2313
Holz's Phenol Gelatin 2314
Stutzer and Hartleb's Urea Gelatin 2315

H_2. Containing additional constituents of unknown chemical composition.

I_1. Cells, tissues or derivatives added.

Park, Williams and Krumwiede's Meat Gelatin 2316
Worth's Glucose Liver Gelatin 2317
Nastinkoff's Egg Yolk Nutrient Gelatin 2318

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* See F_3 next column.

I_2. Body fluids added.
Pergola's Alkaline Blood Gelatin... 2319
Müller's Blood Gelatin............ 2320
Müller's Serum Gelatin........... 2321
I_3. Other animal derivatives added.
A_2. No commercial digest employed. Non-commercial digest used.
Beijerinck's Trypsinized Gelatin... 2322
Jensen's Pepsinized Milk Gelatin... 2323

2235. Will's Basal Salt Peptone Gelatin

Same as medium 583, but solidified by the addition of 10.0% gelatin. The author also solidified the basal solution, see medium 555, by the addition of 10.0% gelatin.

2236. Matzuschita's Basal Peptone Gelatin

Same as variant (b) medium 568, but solidified by the addition of 10.0% gelatin.

References: Kita (1913 p. 446), Matzuschita (1902 p. 286, 288).

2237. Banning's Basal Peptone Gelatin

Same as medium 1535, but solidified by the addition of 70.0 g. gelatin instead of 10.0 g. agar.

2238. Harvey's Basal Neutral Red Peptone Gelatin

Constituents:

1. Water......................	1000.0 cc.
2. Peptone....................	20.0 g.
3. KOH (5.0%)...............	10.0 cc.
4. Gelatin....................	75.0 g.
5. Neutral red (1.0%) (0.5%)..	5.0 cc.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Steam 45 minutes.
(3) Filter.
(4) Add 5.0% of a 20.0% solution of one of the added nutrients, and 0.5% of a 1.0% neutral red solution.

Sterilization: Sterilize for 10 minutes on each of 3 successive days at 100°C.

Use: Cultivation and differentiation of colon group.

Added nutrients: The author added 5.0% of a 20.0% solution of any desired sugar.

Reference: Harvey (1921–22 p. 109).

2239. Weiss' Nährstoff Heyden Gelatin

Constituents:

1. Water......................	1000.0 cc.
2. Gelatin (15.0%)...........	150.0 g.
3. Nährstoff Heyden (0.8%)...	8.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: Bacterial count of water. The author reported that generally an alkaline medium gave a slightly higher bacterial count.

Reference: Weiss (1920 p. 25).

2240. Jacobi's Peptone Gelatin

Constituents:

1. Water......................	1500.0 cc.
2. Meat peptone (Kemmerich's)..................	22.5 g.
3. Peptone (siccum)..........	45.0 g.
4. Gelatin....................	225.0 g.

Preparation:
(1) Add 2 and 3 in 1.
(2) Boil over a free flame for a minute.
(3) Cool to about 50 to 60°C.
(4) Dissolve the gelatin in (3) without further heating.
(5) Correct the acid reaction by the addition of concentrated Na_2CO_3.
(6) Transfer to a large flask.
(7) Add the white of an egg, mix well and steam for ½ hour.
(8) Filter, using compressed air to hasten filtration.
(9) Distribute in 50 to 100.0 cc. lots in flasks.

Sterilization: Place in flowing steam for 10 minutes on 3 successive days.

Use: General culture medium. Other investigators used similar media for specific purposes as indicated below.

Variants:
(a) Jacobi stated that the medium might be diluted with distilled water when ready for use.
(b) Matzuschita dissolved 10.0 g. of Koch's peptone in 1000.0 cc. water and solidified with gelatin. He cultivated spore forming bacilli on this medium.
(c) Lieske isolated iron bacteria, *Leptothrix ochracea* on a medium made slightly alkaline by the addition of KOH containing 100.0 g. of gelatin and 0.25 g. manganese peptone per 1000.0 cc. of peat water.
(d) Weiss used 1.0% peptone instead of

0.8% Nährstoff-Heyden in the preparation of medium 2239.

- (e) Levine and Carpenter studied the liquefaction of gelatin on a medium prepared as follows:
  - (1) Add 1.0 g. Difco peptone, 35.0 g. Difco gelatin to 1000.0 cc. distilled water and heat, at 60–65°C. until all material is in solution.
  - (2) Heat in double boiler for 15.0 minutes.
  - (3) Adjust to pH = 8.0.
  - (4) Make up volume.
  - (5) Heat 15.0 more minutes.
  - (6) Filter thru cotton flannel.
  - (7) Distribute in 25.0 cc. quantities.
  - (8) Sterilize at 15.0 pounds for 15.0 minutes.

References: Jacobi (1888 p. 539), Matzuschita (1902 p. 286), Lieske (1919 p. 417), Weiss (1920 p. 24), Levine and Carpenter (1923 p. 298).

2241. Metchnikoff's Peptone Gelatin (Thoinot and Masselin)

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	10.0 g.
4. Gelatin	20.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Enrichment of Bacillus of Asiatic cholera. Sergent cultivated *B. zopfi* on a similar medium.

Variants:

- (a) Wherry gave the following method of preparation:
  - (1) Dissolve 200.0 g. Gold Label gelatin, 10.0 g. Witte's peptone and 5.0 g. NaCl in 1000.0 cc. of water by boiling.
  - (2) Divide in two 500.0 cc. portions.
  - (3) Adjust one portion to +1 reaction to phenolphthalein with normal NaOH. To the other portion add an equal volume of normal Na_2CO_3.
  - (4) Cool to 40°C., add whites of three eggs to each.
  - (5) Boil for 3 minutes.
  - (6) Filter thru cotton and distribute.
  - (7) Sterilize in Arnold for 20 minutes on each of 3 successive days.
  - (8) Final reaction of NaOH portion +1.7.
    Final reaction of Na_2CO_3 portion +1.8.
- (b) Sergent cultivated *Bact. zopfi* on a medium prepared by dissolving 5.0 g. NaCl, 10.0 g. peptone and 80.0 g. gelatin in 1000.0 cc. distilled water.
- (c) Roux and Rochaix dissolved 10.0 g. peptone, 0.5 g. NaCl and 20.0 g. gelatin in 1000.0 cc. water and made the reaction slightly alkaline (indicator not specified).
- (d) Besson prepared the medium as follows:
  - (1) Dissolve 10.0 g. Chapoteaut's peptone, 5.0 g. NaCl, and 20.0 g. gelatin (extra white) in 1000.0 cc. water.
  - (2) Make slightly alkaline by the addition of soda solution.
  - (3) Heat 5 minutes at 115°C.
  - (4) Filter thru paper.
  - (5) Distribute as desired.
  - (6) Sterilize at 110 to 115°C.

References: Thoinot and Masselin (1902 p. 392), Wherry (1905 p. 320), Sergent (1906 p. 1015), Roux and Rochaix (1911 p. 109), Besson (1920 p. 30).

2242. Mortensen's Peptone Gelatin

Same as medium 560 but solidified by the addition of gelatin.

2243. Rossi's Salt Peptone Gelatin

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone	20.0 g.
3. NaCl	5.0 g.
4. Gelatin	100.0 g.
5. Na_2CO_3	0.6 g.
6. K_2CO_3	0.9 g.
7. NaOH or KOH	2.0 to 4.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.

Sterilization: Not specified.

Use: To be used in water examination.

Reference: Rossi (1893 p. 69).

2244. Metchnikoff's Nitrate Peptone Gelatin (Brau)

Constituents:

1. Water	
2. Gelatin	2.0 g.

3. Peptone (dry)	1.0 g.
4. NaCl	1.0 g.
5. KNO_3	0.1 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in sufficient quantity of water.

Sterilization: Method not given.

Use: Isolation of cholera vibrio. The author added 200.0 cc. of the water under investigation to each flask.

Variants: Pergola solidified medium 549 by the addition of 10.0 to 15.0% gelatin.

References: Brau (1905 p. 813), Pergola (1911 p. 85).

2245. Lichtenstein's Cenovis Gelatin

Same as medium 518 but solidified by the addition of gelatin.

2246. Utz's Glucose Peptone Gelatin

Constituents:

1. Water	1000.0 cc.
2. Gelatin	100.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Glucose	20.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Neutralize.
(3) Mix with egg white.
(4) Shake.
(5) Boil.
(6) Filter.
(7) Add 20.0 g. glucose.

Sterilization: Not specified.

Use: Cultivation of organisms found in milk. d'Herelle cultivated the Coccobacillus of the grasshopper (*Coccobacillus acridiorium*).

Variants: d'Herelle prepared a similar medium as follows:

(1) Dissolve 40.0 g. Chapoteaut's peptone, 5.0 g. NaCl, 30.0 g. gelatin and 5.0 g. glucose in 1000.0 water by boiling.
(2) Make slightly alkaline.
(3) Filter.
(4) Distribute in bottles.
(5) Plug with cotton and cover the cotton with a layer of parchment paper.
(6) Sterilize at 120° for 30 minutes.

He reported that the virulence of the culture was maintained for about 15 days in this medium. When a culture was powdered due to the gelatin content of the medium, it stuck to the plants very well.

References: Utz (1903–04 p. 610), d'Herelle (1914 p. 310).

2247. Beijerinck's Glucose Peptone Gelatin

Constituents:

1. Water	100.0 cc.
2. Glucose	5.0 g.
3. Peptone	5.0 g.
4. Gelatin	10.0 g.
5. Potassium biphosphate	0.02 g.

Preparation: Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Cultivation of vinegar bacteria producing pigment. The author reported that the gelatin was first liquefied and then coagulated.

Reference: Beijerinck (1911 p. 174).

2248. Pfeiler and Lentz's Ringer Solution Peptone Gelatin

Same as medium 1551 but solidified by the addition of 15.0% gelatin instead of agar.

2249. Vierling's Glucose Peptone Gelatin

Constituents:

1. Water	1000.0 cc.
2. K_2HPO_4	1.0 g.
3. $CaCl_2$	0.1 g.
4. $MgSO_4$	0.1 g.
5. $FeCl_3$	trace
6. NaCl	trace
7. Gelatin	100.0 g.
8. Peptone	10.0 g.
9. Dextrose	10.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7, 8 and 9 in 1.
(2) Adjust to a slightly alkaline reaction by the addition of soda.

Sterilization: Not specified.

Use: To study proteolytic action, liquefaction by mycobacteria. The author reported that growth was good, but liquefaction did not take place. Same results if glucose be omitted.

Reference: Vierling (1920 p. 203).

2250. Sear's Glucose Peptone Gelatin

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.

3. NaCl	5.0 g.
4. Gelatin	50.0 g.
5. Glucose (1.0%)	10.0 g.
6. $CaCO_3$	

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Clear with white of egg.

(3) Neutralize to phenolphthalein.

(4) Filter till clear.

(5) Add 1.0% glucose (glucose may be omitted).

(6) Distribute both portions into 300.0 cc. flasks, 200.0 cc. per flask.

Sterilization: Sterilize at 15 pounds for 10 minutes.

Use: To study nitrogen metabolism. Ammonia determined by Folin's method, amino acid by Van Slyke's micro method and total nitrogen by Kjeldahl-Gunning-Arnold method.

Reference: Sears (1916 p. 126).

2251. Utz's Lactose Peptone Gelatin

Same as medium 2246 but used 20.0 g. lactose instead of glucose.

2252. Molisch's Sucrose Peptone Gelatin (Smith)

Constituents:

1. Water	1000.0 cc.
2. Gelatin	100.0 g.
3. Sucrose	20.0 g.
4. Peptone	10.0 g.
5. K_2HPO_4	0.25 g.
6. $MgSO_4$	0.25 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Make feebly alkaline by the addition of NaOH.

Sterilization: Not specified.

Use: Cultivation of luminous bacteria. Smith reported that on this medium the bacteria grew feebly and were non-luminous until NaCl or some equivalent material was added (3.0%).

Reference: Smith (1905 p. 198).

2253. Trommsdorff's Sucrose Peptone Gelatin

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte) (0.5%)	5.0 g.
3. Sucrose (0.5%)	5.0 g.
4. Gelatin (8.0%)	80.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of Leptomitus.

Reference: Trommsdorff (1918 p. 65).

2254. Molisch's Dextrin Peptone Gelatin

Constituents:

1. Moldau water	1000.0 cc.
2. Gelatin	100.0 g.
3. Peptone	5.0 g.
4. Dextrin	5.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of purple bacteria.

Reference: Molisch (1907 p. 11).

2255. Matzuschita's Glycerol Peptone Gelatin

Constituents:

1. Water	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	5.0 g.
4. Gelatin	80.0 to 100.0 g.
5. Glycerol	60.0 g.

Preparation:

(1) Prepare ordinary nutrient gelatin using water instead of bouillon. (Peptone and NaCl, in the amounts indicated, were considered to be the constituents of nutrient gelatin. Exact amount of gelatin not specified in the article either.)

(2) Add 6.0% glycerol to (1).

Sterilization: Not specified.

Use: Cultivation of mammalian and chicken tubercle bacilli.

Reference: Matzuschita (1899 p. 128).

2256. Molisch's Glycerol Peptone Gelatin

Constituents:

1. Moldau water	1000.0 cc.
2. Gelatin	100.0 g.
3. Peptone	5.0 g.
4. Glycerol	5.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Not specified.

Use: Cultivation of purple bacteria.

Reference: Molisch (1907 p. 11).

2257. Freudenreich and Jensen's Lactate Peptone Gelatin (Boekhout and Ott de Vries)

Same as medium 644 but solidified by the addition of 10.0% gelatin.

Reference: Boekhout and Ott de Vries (1918 p. 130).

2258. Matzuschita's Glucose Glycerol Peptone Gelatin

Constituents:

1. Water	1000.0 cc.
2. Gelatin (10.0%)	100.0 g.
3. NaCl (0.5%)	5.0 g.
4. Peptone (1.0%)	10.0 g.
5. Glycerol (6.0%)	60.0 g.
6. Glucose (2.0%)	20.0 g.

Preparation:

(1) Dissolve gelatin in water.
(2) Neutralize (indicator not specified).
(3) Add 0.5% NaCl, 10.0% peptone, 6.0% glycerol and 2.0% glucose.
(4) Filter.

Sterilization: Method not given.

Use: Cultivation of mammalian and chicken tubercle bacilli. The author reported that mammalian type gave gray white flakes which sank to the bottom of the liquid at 37°C. In gelatin a gray white membrane formed on top. Chicken type gave large flakes and a gray white precipitate at the bottom. A membrane also formed on the surface.

Reference: Matzuschita (1899 p. 128).

2259. Dombrowski's Glucose Peptone Gelatin

Same as medium 631 but solidified by the addition of 10.0% gelatin.

2260. Beijerinck's Asparagin Peptone Gelatin

Constituents:

1. Water	900.0 cc.
2. Sucrose	10.0 g.
3. Asparagin	2.0 g.
4. Peptone	8.0 g.
5. Gelatin	80.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Adjust (1) to neutral or slightly acid. Indicator not specified.

Sterilization: Method not specified.

Use: To study liquefaction of gelatin and pigment production by Chlorococcum. Chlorococcum did not liquefy the gelatin.

Reference: Beijerinck (1890 p. 461).

2261. Remy's Phenolated Lactose Peptone Gelatin

Constituents:

1. Distilled water	1000.0 cc.
2. Asparagin	6.0 g.
3. Oxalic acid	0.5 g.
4. Lactic acid	0.15 g.
5. Citric acid	0.15 g.
6. Bisodium phosphate	5.0 g.
7. MgSO$_4$	2.5 g.
8. K$_2$SO$_4$	1.25 g.
9. NaCl	2.0 g.
10. Gelatin	120.0 to 150.0 g.
11. Peptone (Witte's)	30.0 g.
12. Lactose	
13. Phenol	

Preparation:

(1) Grind 6, 8 and 9 in a mortar.
(2) Dissolve (1), 2, 3, 4 and 5 in 1.
(3) Add 30.0 g. peptone (Witte or Grübler's) to (2) and autoclave under pressure for 15 minutes.
(4) Pour the hot solution into a flask containing 120.0 to 150.0 g. of gelatin. Shake thoroly until solution is complete.
(5) Make slightly alkaline by the addition of soda.
(6) Heat in the autoclave under pressure for 15 minutes at 110°C.
(7) Acidify by the addition of 0.5N H$_2$SO$_4$ so that the acidity of 10.0 cc. of gelatin will be neutralized by 0.2 cc. of a 0.5 N soda solution.
(8) Steam again for 8 or 10 minutes.
(9) Filter.
(10) Retest the reaction by adding 10.0 cc. of gelatin to 100.0 cc. water and several drops of phenolphthalein. Add 0.5 N soda solution drop by drop until a red coloration appears. 0.2 cc. of the soda solution should be required.
(11) Add 2.5 g. MgSO$_4$.
(12) Distribute in 10.0 cc. lots.
(13) Just before use, add 1.0 cc. of a 35.0% lactose solution and 0.1 cc. of a 2.5% phenol solution to each tube of sterile (12).

Sterilization: Sterilize by heating on 3 successive days.

Use: Isolation of typhoid bacillus.

Reference: Remy (1900 p. 561).

2262. Scholz and Krause's Urea Peptone Gelatin

Constituents:

1. Water	1000.0 cc.
2. Gelatin	30.0 g.
3. Peptone	5.0 g.
4. Urea	20.0 g.
5. Sodium or ammonium urate	3.0 g.
6. $(NH_4)_2CO_3$	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Adjust the reaction to slight alkalinity.

Sterilization: Method not specified.

Use: Cultivation of colon-typhoid group. The author reported that colon and typhoid colonies could not be distinguished on this medium.

Reference: Scholz and Krause (1900 p. 431).

2263. Beijerinck's Peptone Malt Gelatin

Constituents:

1. Malt infusion	890.0 cc.
2. Glucose	29.0 g.
3. Peptone	0.5 g.
4. Asparagin	0.5 g.
5. Gelatin	80.0 g.

Preparation:

(1) Method of preparation of malt infusion not given.

(2) Dissolve 2, 3, 4 and 5 in (1).

(3) Adjust (1) to neutral or slightly acid. Indicator not specified.

Sterilization: Method not given.

Use: To study liquefaction of gelatin and pigment production by chlorococcum. The author reported that chlorococcum did not liquefy the gelatin.

Reference: Beijerinck (1890 p. 461).

2264. d'Herelle's Peptone Potato Gelatin

Constituents:

1. Water	1000.0 cc.
2. Potatoes, grated	10.0 g.
3. Peptone	5.0 g.
4. NaCl	5.0 g.
5. Gelatin	20.0 g.
6. Glucose	5.0 g.

Preparation:

(1) Heat 2, 3, 4, 5 and 6 to boiling in 1 and boil (time not specified).

(2) Make slightly alkaline.

(3) Filter.

(4) Distribute in bottles.

(5) Plug with cotton and cover the cotton with a layer of parchment paper.

Sterilization: Sterilize at 120° for 30 minutes.

Use: Cultivation of Coccobacillus of the grasshopper, *Coccobacillus acridiorium* This medium was used in field work. See also variant of medium 2246.

Reference: d'Herelle (1914 p. 310).

2265. Elsner's Peptone Potato Gelatin (Heinemann)

Constituents:

1. Water	1000.0 cc.
2. Potato	1.0 lb.
3. Peptone (1.0%)	10.0 g.
4. KI (1.0%)	10.0 g.
5. Gelatin (10.0%)	100.0 g.

Preparation:

(1) Grate 1 pound of potatoes with 1 liter of water.

(2) Express the juice.

(3) Filter for 24 hours (method not given).

(4) Dissolve 1.0% peptone, 1.0% KI and 10.0% gelatin in (3).

Sterilization: Not specified.

Use: Cultivation of colon typhoid group.

Reference: Heinemann (1905 p. 129).

2266. de Rossi's v. Faba Peptone Gelatin

Same as medium 1623 but containing 10.0% gelatin instead of 1.5% agar.

2267. Molisch's Pea Gelatin (Löhnis)

Constituents:

1. Water	1000.0 cc.
2. Peas	
3. Peptone, manganese (0.25%)	2.5 g.
4. Gelatin	

Preparation:

(1) Prepare a pea infusion with boiling water (method not given).

(2) Add 0.25% manganese peptone to (1).

(3) Solidify with agar.

Sterilization: Not specified.

Use: Cultivation of iron bacteria.

Reference: Löhnis (1913 p. 116).

2268. Molisch's Peat Gelatin

Constituents:

1. Water	1000.0 cc.

2. Peat	
3. Peptone, manganese	0.25 g.
4. Gelatin	100.0 g.

Preparation:

(1) Boil a piece of peat brick the size of a fist in a liter of water to prepare the peat infusion.

(2) Dissolve 2 and 3 in (1).

(3) Make slightly alkaline by the addition of NaOH.

Sterilization: Not specified.

Use: Cultivation of iron bacteria, leptothrix.

Reference: Molisch (1910 p. 39).

2269. Deycke's Peptone Alkali Albumin Gelatin

Constituents:

1. Water	1200.0 cc.
2. KOH (3.0%)	36.0 g.
3. Peptone	1.0%
4. Veal	1000.0 g.
5. Gelatin	10.0%
6. NaCl	1.0%

Preparation:

(1) Digest 1000.0 g. finely ground and fat-free veal in 1200.0 cc. of 3.0% KOH in flask for 2 days at 37°C.

(2) Heat one hour on water bath 60–70°C.until all protein dissolves.

(3) Add HCl carefully to precipitate albuminate.

(4) Collect on filter.

(5) Suspend in distilled water and add concentrated soda solution until alkaline reaction brings part into solution.

(6) Dissolve by prolonged heating in steam.

(7) Correct reaction to weak alkalinity.

(8) Determine % dry weight by drying 100.0 cc. sample at 100°C.

(9) Dilute (8) so that there is about 3.0% dry material present.

(10) Add 1.0% peptone, 1.0% NaCl and 10.0% gelatin.

(11) Dissolve and neutralize by Dahmen's method using 0.33% soda solution.

Sterilization: Sterilize on 3 successive days for 10 minutes each day in streaming steam.

Use: Isolation of cholera bacilli from stools. The author reported the medium eliminated practically all other intestinal organisms.

References: Deycke (1893 p. 888), (1895 p. 243), Klimmer (1923 p. 220).

2270. Kotlar's Pancreas Gelatin

Constituents:

1. Water	400.0 cc.
2. Pancreas (calf)	200.0 g.
3. Peptone (2.0%)	2.0 g.
4. NaCl (1.0%)	1.0 g.
5. Gelatin (20.0%)	20.0 g.

Preparation:

(1) Add a little water to 200.0 g. of small fat and connective tissue free pieces of calf pancreas and mince to a pulp.

(2) Add 200.0 cc. of water to (1).

(3) Filter thru a clay filter.

(4) Prepare a watery solution of 20.0% gelatin containing 2.0% peptone and 1.0% NaCl.

(5) Mix 100.0 cc. of sterile (4) and 100.0 cc. of sterile (3).

Sterilization: Sterilize (2) by filtering thru a clay filter (see step (3) above). Method of sterilization of (4) not given.

Use: Show effect of pancreas extract on growth of *Bact. coli*, *Bact. typhosus*, cholera bacillus, anthrax bacillus and *Staphylococcus pyogenes aureus*. Author reported that the organisms were inhibited by the extract.

Reference: Kotlar (1895 p. 147).

2271. Harde's Tissue Gelatin

Constituents:

1. Nutrient gelatin.
2. Beef or veal.

Preparation: (1) Add a 2 cc. cube of beef or veal to 20.0% gelatin just before sterilization.

Sterilization: Method not given.

Use: To obtain anaerobes and aerobes from exudates.

Reference: Harde (1917 p. 661).

2272. Pergola's Nitrate Alkaline Blood Gelatin

Constituents:

1. Distilled water	1000.0 cc.
2. Peptone	10.0 g.
3. NaCl	10.0 g.
4. Na_2CO_3	0.2 g.
5. KNO_3	0.1 g.

6. Gelatin	100.0 to 150.0 g.
7. Blood	
8. KOH (normal)	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Filter.
(3) Mix blood with equal amounts of N/1 KOH.
(4) Steam (3) for 30 minutes to sterilize.
(5) Mix 30.0 parts sterile (4) with 70.0 parts sterile (2).
(6) Pour into sterile Petri dishes.

Sterilization: Method of sterilization of (2) not given. See step (4) for sterilization of alkaline blood mixture.

Use: Enrichment medium for cholera vibrio.

Reference: Pergola (1911 p. 85).

2273. Matzuschita's Peptone Milk Gelatin

Constituents:

1. Milk	1000.0 cc.
2. Gelatin	100.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.

Preparation: (1) Dissolve 2, 3 and 4 in 1.

Sterilization: Method not specified.

Use: Cultivation of mammalian and chicken tubercle bacilli. The author reported that chicken type grew slightly more luxuriantly than did the mammalian types. Utz cultivated organisms found in milk on a similar medium.

Variants: Utz prepared a similar medium as follows:

(1) Dissolve 100.0 g. gelatin and 10.0 g. peptone in 1000.0 cc. milk.
(2) Neutralize.
(3) Mix with egg white.
(4) Boil one hour.
(5) Filter.
(6) Sterilization not specified.

References: Matzuschita (1899 p. 128), Utz (1903–04 p. 610).

2274. Raskin's Whey Peptone Gelatin

Constituents:

1. Whey	1000.0 cc.
2. Gelatin (6.0 to 10.0%)	60.0 to 100.0 g.
3. Peptone (1.0%)	10.0 g.

Preparation:

(1) Heat 1000.0 cc. of milk in a porcelain dish at 60 to 70°C.
(2) Add 60.0 to 100.0 g. (6.0 to 10.0% gelatin and heat until the gelatin has become melted.
(3) Boil until the casein is completely coagulated.
(4) Pass thru four folds of linen cloth separating the casein and liquid.
(5) The reaction of the fluid is slightly acid.
(6) Pour into a tall glass cylinder and place in a warm thermostat.
(7) After about 20 or 30 minutes two layers are formed, the lower layer being transparent and fat free, the upper layer yellowish white, containing the fat.
(8) Allow to cool and remove the fat by means of a spoon.
(9) Heat the fat free portion to boiling and add 1.0% peptone.
(10) Add soda to neutralize the reaction.
(11) Filter until clear thru a paper in a hot water funnel.

Sterilization: Not specified.

Use: Cultivation of pathogenic organisms.

Variants:

(a) Raskin added 0.5% NaCl.
(b) Klimmer prepared the medium as follows:
  (1) Coagulate the casein from milk by heating at 40°C. in the presence of rennet.
  (2) Separate the whey from the casein.
  (3) Dissolve 1.0% peptone, 0.5% NaCl and gelatin to solidify in the whey.
  (4) Sterilization not specified.
(c) Cunningham gave the following method of preparation:
  (1) Warm 2 liters of clean fresh skim milk in a large pot at 37°C.
  (2) Add sufficient rennet to curdle.
  (3) Allow to settle for 10 minutes.
  (4) Break the curd into large pieces by means of a stirring rod.
  (5) Heat to 80°C. to contract the clot and to express the whey.
  (6) Strain thru a cheese cloth.
  (7) Add 1.0% peptone and 0.5% NaCl.
  (8) Steam for 30 minutes.
  (9) Neutralize to turmeric paper.
  (10) Steam for one hour.
  (11) Dissolve 10.0% gelatin in (10).
  (12) Filter thru paper until clear.
  (13) Add 1.0% Andrades indicator.
  (14) Sterilize intermittently in steam.

References: Raskin (1887 p. 358), Klimmer (1923 p. 172), Cunningham (1924 p. 102).

2275. Appel's Peptone Whey Gelatin

Constituents:

1. Water to	1000.0 cc.
2. Whey	
3. Gelatin	100.0 g.
4. Peptone	10.0 g.
5. NaCl	5.0 g.

Preparation:

(1) Heat 1 liter of centrifuged milk to 40°C.
(2) Add a little rennet and allow to stand until coagulation takes place.
(3) Heat for about 15 minutes on a boiling water bath.
(4) Pour thru a straining cloth, stirring with a porcelain spatula to cause a separation of the whey and coagulum.
(5) The yellow colored whey is brought to 1000.0 cc. by the addition of water.
(6) Add 100.0 g. gelatin, 10.0 g. peptone and 5.0 g. NaCl. If one works fast the solution is warm enough to dissolve the gelatin.
(7) Mix well and autoclave at 105° for 30 minutes.
(8) Immediately after removing filter thru paper and without allowing the gelatin to solidify, distribute into sterile tubes.

Sterilization: Sterilize once more at 105° for 30 minutes.

Use: Cultivation of organisms found in milk. The author reported that the gelatin had a high melting point.

Reference: Appel (1899 p. 763).

2276. Meier's Whey Peptone Gelatin

Constituents:

1. Water	500.0 cc.
2. Whey (goat milk)	500.0 cc.
3. Gelatin	110.0 g.
4. Peptone (Witte)	10.0 g.
5. NaCl	5.0 g.

Preparation:

(1) Dissolve 3, 4 and 5 in 1.
(2) Mix (1) with 500.0 cc. of whey from goat milk.
(3) Neutralize by the addition of KOH. Add KOH until turmeric paper is turned quite weakly brownish red.

Sterilization: Not specified.

Use: To determine bacterial counts of milk and milk products.

Reference: Meier (1918 p. 436).

2277. Wigger's Whey Gelatin

Constituents:

1. Whey, goat.
2. Gelatin.
3. Peptone.

Preparation:

(1) Add 10 to 12 drops of rennet extract of a strength of 1:10,000 to 1 liter of fresh skim milk heated to 30 or 35°C. This coagulates the casein in 30 or 45 minutes to a gelatinous mass.
(2) Heat at 55 to 60°C. and the casein separates out in large lumps leaving a clear whey.
(3) Mix sterile (2) with an equal amount of water.
(4) For the preparation of gelatin use (3) instead of meat water, omit the NaCl and proceed in the usual manner. (It was assumed that peptone was added.)

Sterilization: Sterilize the whey in the autoclave at one-half atmosphere additional pressure for 45 minutes. Final sterilization not specified.

Use: Cultivation of milk bacteria.

Variants: The author added lactic acid to the undiluted whey and heated to 80 or 90°C. to precipitate the albumin.

Reference: Wigger (1914 p. 3).

2278. Piorkowski's Peptone Urine Gelatin

Constituents:

1. Urine	1000.0 cc.
2. Peptone	5.0 g.
3. Gelatin	100.0 to 120.0 g.

Preparation:

(1) Add 100.0 cc. urine and 0.5 g. peptone to a flask and plug with cotton.
(2) Steam for 15 minutes in a steamer.
(3) Dissolve 10.0 to 12.0% gelatin in (2).
(4) Filter using a hot water funnel or in a steamer.
(5) Distribute into 10.0 cc. lots.

Sterilization: Sterilize on each of two successive days for 10 to 15 minutes in the steamer.

Use: Differentiation of *Bact. coli, commune* and *Bacillus typhi abdominalis.* Urine should be fresh, clear, bright yellow and of acid reaction. A urine of specific gravity of 1.012 gives the best medium.

Variants: Piorkowski prepared similar media as follows:

(a) He reported that on the following medium *Bact. coli commune* colonies after 20 hours, under the microscope, were round, yellow fine grained and edges were well defined. *Bact. typhi abd.* gave thread like colonies. Do not incubate at a temperature lower than 22°C.

  (1) Allow normal urine to stand for 2 days. Specific weight of the urine to be 1.020. During this time the reaction has become alkaline.
  (2) Add 0.5% peptone and 3.3% gelatin to (1).
  (3) Boil for an hour in a water bath.
  (4) Filter at once without the use of heat.
  (5) Distribute into test tubes and plug with cotton.
  (6) Sterilize in the steamer for 15 minutes at 100°C. and on the following day sterilize at 100°C. for 10 minutes.
  (7) Incubate at 22°C.

(b) On the following medium typhoid colonies were small and colorless, with 4 to 6 turft like runners. *Bact. coli* colonies were large and yellow without the runners.

  (1) Dissolve 0.5% peptone and 3.3% gelatin in alkaline urine having a specific gravity of 1.020.
  (2) Allow to stand at 22° for 16 to 20 hours.
  (3) Sterilization not specified.

References: Piorkowski (1896 p. 687), (1899 p. 145), (1916–17 p. 259).

2279. Heller's Urine Peptone Gelatin

Same as medium 745 but solidified by the addition of 10.0% gelatin.

2280. Schultz's Peptone Infusion Gelatin

Constituents:

1. Water	1300.0 cc.
2. Meat	500.0 g.
3. Peptone (siccum)	10.0 g.
4. NaCl	5.0 g.
5. Gelatin	50.0 to 100.0 g.

Preparation:

(1) Place 500.0 g. of the best quality meat, without fat or tendons, in a glass container fitted with a lid.
(2) Pour 1300.0 cc. distilled water over the meat.
(3) Store in a cool place until the next day.
(4) Filter thru four thicknesses of cloth and press the remaining meat to obtain as much fluid as possible.
(5) Pour the filtrate into a kettle, add 10.0 g. peptone (siccum) 5.0 g. NaCl and the whites of two eggs, beaten up in two or three volumes of water.
(6) Boil over a gas flame for 15 minutes.
(7) Adjust to faint alkalinity, using phenolphthalein as an indicator, and the end point being a faint red color.
(8) Pour into an iron kettle, add 100.0 cc. distilled water, boil strongly for 5 minutes and filter.
(9) Dissolve 50.0 to 100.0 g. of gelatin in (8).
(10) Add 200.0 cc. distilled water to (9).
(11) Cool to 40°C. and add the whites of two eggs beaten up in 2 or 3 volumes of water.
(12) Boil strongly for 10 to 15 minutes.
(13) Filter thru four layers of cloth and then thru a hot water funnel.

Sterilization: Sterilize for 30 minutes on each of 3 successive days in a steamer.

Use: General culture medium.

Variants:

(a) Acosta and Grande (1892) (Sentinon 1893).

  (1) Add to 1000.0 of tendon free lean meat a double weight of water.
  (2) Boil (time not specified).
  (3) Skim and strain.
  (4) Place once more on the fire and and 0.5% peptone and 0.25% NaCl and add water to bring up to the original volume.
  (5) Add 16.0 to 18.0% gelatin.
  (6) Pour the solution in a clay or glass container that is twice as high as wide.
  (7) Autoclave at 105° at 0.5 atmosphere pressure for 15 minutes.
  (8) Release the pressure and allow the mixture to stand for 24 hours.

(9) Remove from the autoclave, and loosen the solidified gelatin from the walls of the container.
(10) Upset the gelatin on a filter paper.
(11) Cut off the turbid upper layer of the gelatin cylinder with a thread or wire.
(12) Cut the clear gelatin into pieces, place into a flask and melt.
(13) Distribute into test tubes.
(14) Sterilize by the discontinuous method, (details not given).

(b) Frothingham (1895).
(1) Add one pound of finely chopped lean meat to 1000.0 cc. water and allow to stand from 12 to 24 hours in a cool place.
(2) Strain thru a cheese cloth or coarse towel and squeeze in a meat press or by twisting the ends of the cloth until 1000.0 cc. of the meat juice is obtained. Make up to 1000.0 cc. by the addition of water if necessary.
(3) Dissolve 5.0 g. NaCl and 10.0 g. dried peptone in (2).
(4) Add 100.0 g. gelatin to (3).
(5) Heat in a water bath to 50° until the gelatin is dissolved.
(6) Make slightly alkaline.
(7) Boil for 45 minutes to 60 minutes.
(8) Filter.

(c) Forster (1897).
(1) Prepare Loeffler's bouillon.
(2) Sterilize (1) method not given.
(3) Heat to 60°C. and dissolve the necessary or required amount (exact amount not given) of gelatin in (1).
(4) Make slightly alkaline and cool a little.
(5) Add the white of an egg.
(6) Place the kettle containing (5) in boiling water, and stir with a spoon to insure equal heating.
(7) Adjust the reaction once more and heat to 100°C. for 15 minutes. Leave the lid loosely on the kettle.
(8) Heat a water funnel to 60°C. (not higher) and filter quickly.
(9) Distribute the filtrate into sterile culture tubes.
(10) Heat in boiling water or in flowing steam at 100°C. for 17 to 20 minutes.

(d) Jensen (1898) cultivated denitrifying bacteria on the following medium:
(1) Prepare an infusion from 500.0 g. meat and 1000.0 cc. of water.
(2) Dissolve 5.0 g. NaCl, 10.0 g. peptone and 200.0 g. of gelatin in (1).
(3) Adjust the reaction to a slight alkalinity by adding soda.
(4) Method of sterilization not specified.

(e) Committee A. P. H. A. (1899).
(1) Macerate one part finely chopped lean meat with 2 parts distilled water in an ice box for 18 to 24 hours, stirring constantly.
(2) Strain while cold thru a fine cloth.
(3) Add 1.0% peptone and 0.5% NaCl to the filtrate. Heat until solution is complete.
(4) Add NaOH until the reaction is slightly alkaline (practically neutral) to phenolphthalein.
(5) Heat on a water bath for 30 minutes and boil 5 minutes over a free flame.
(6) Filter while hot thru paper or cotton and cloth, and dissolve 10.0% gelatin in the filtrate.
(7) Add normal HCl to the filtrate to obtain the desired reaction. If the medium is clear distribute in tubes or flasks. If not clear, clarify by adding the whites of one egg to the medium cooled to 50 or 60° and boil vigorously. Filter.
(8) Sterilize either by the fractional or continuous method.

(f) Migula (1901).
(1) Mix 500.0 g. of finely chopped lean beef with one liter of water, and allow to stand in the ice box for 12 to 24 hours.
(2) Press the liquid thru a towel and make up the volume to one liter.
(3) Boil in the steamer cooker for 30 minutes.
(4) The infusion may be boiled for an hour before removing the meat and then filtered thru paper. If the liquid is still red, boil again for 15 minutes.
(5) Filter when cold to remove any fat.
(6) Heat to boiling and add 0.5% NaCl, 1.0% Witte's peptone and

10.0% gelatin (1.0 to 2.0% glucose may be added).
- (7) Neutralize carefully by the addition of concentrated Na_2CO_3 until litmus paper is colored violet.
- (8) Add soda solution as desired. Generally 10.0 cc. of 15.0% soda solution is added per liter.
- (9) Boil for 30 minutes in the steamer.
- (10) Filter thru paper.
- (11) The gelatin should be perfectly clear. If not cool to 40°C., and mix thoroly with the white of an egg.
- (12) Boil again for a short time.
- (13) Filter.
- (14) Distribute in tubes or flasks.
- (15) Sterilize on 2 successive days in flowing steam for 20 minutes each day, and for 10 minutes on the third day, or for 15 minutes on each of 3 successive days. Cool quickly after each heating.

(g) Roux (Thoinot and Masselin 1902).
- (1) Macerate 500.0 g. of finely chopped beef in a liter of water for several hours.
- (2) Pass thru a linen cloth and express the juice from the meat.
- (3) Add 10.0 g. peptone, 5.0 g. NaCl and 100.0 g. of light colored gelatin to (2).
- (4) Heat in a water bath not above 60°C. to dissolve the gelatin.
- (5) Add soda to make slightly alkaline.
- (6) Heat in steam at 100° for an hour.
- (7) Filter.
- (8) If not clear, cool to 50°C. and add the white of an egg and heat again to 100°C.
- (9) Filter while hot thru wet Chardin paper.
- (10) Tube in sterile tubes.
- (11) Sterilize for 15 minutes on each of 3 successive days at 100°C.

(h) Thoinot and Masselin (1902).
- (1) Macerate 500.0 g. of lean beef with a liter of water.
- (2) Express the juice and pass thru a linen cloth.
- (3) Boil several minutes.
- (4) Filter thru moistened filter paper.
- (5) Add 10.0 g. peptone, 5.0 g. NaCl and 100.0 g. gelatin.
- (6) Heat over a small flame or on a water bath until the gelatin is completely dissolved.
- (7) Make slightly alkaline.
- (8) Heat for 5 minutes at 113–114°C. in the autoclave and filter while hot thru Chardin paper.
- (9) Distribute as desired.
- (10) Sterilize at 112°C. for 25 minutes.

(i) Frost (1903).
- (1) Remove all the fat and connective tissue from 500.0 g. beef and mince or use hamburger steak.
- (2) Add 1 liter of distilled water to (1), shake thoroly and set in the ice chest for 12 to 24 hours.
- (3) Squeeze thru a cloth and add enough distilled water to make one liter.
- (4) Add 1.0% peptone (Witte) 0.5% NaCl and from 10.0 to 15.0% of the best gold label sheet gelatin. (Add 10.0% gelatin in winter and 15.0% gelatin in summer.)
- (5) Weigh the solution and the vessel.
- (6) Heat until solution is complete.
- (7) Neutralize to phenolphthalein.
- (8) Boil 5 minutes and restore the weight lost by the addition of distilled water.
- (9) Test the reaction, and readjust if necessary.
- (10) Boil until the albumin coagulates and floats in the clear liquid.
- (11) Filter thru cotton supported on a coil of wire using a suction pump to hasten filtration.
- (12) Add 5.0 cc. (0.5%) of a normal HCl solution.
- (13) Tube.
- (14) Sterilize in the steamer for 30 minutes on 3 consecutive days or in the autoclave at 110° for 15 minutes.

(j) Frost (1903).
- (1) Remove all the fat and connective tissue from 500.0 g. of beef and mince, or use hamburger steak.
- (2) Add 1 liter of distilled water.
- (3) Place in a vessel for cooking and cook for 30 minutes at about 70°C.
- (4) Filter thru paper and make up to 1 liter.
- (5) Add 1.0% peptone (Witte) 0.5%

NaCl and from 10.0 to 15.0% of the best gold label sheet gelatin. (Add 10.0% gelatin in winter and 15.0% gelatin in summer.)
(6) Weigh the solution and the vessel.
(7) Heat until solution is complete.
(8) Neutralize to phenolphthalein.
(9) Boil 5 minutes and restore the weight lost by the addition of distilled water.
(10) Test the reaction and readjust if necessary.
(11) Boil until the albumin coagulates and floats in the clear fluid.
(12) Filter thru cotton supported on a coil of wire using a suction pump to hasten filtration.
(13) Add 5.0 cc. (0.5%) of a normal HCl solution.
(14) Tube.
(15) Sterilize in the steamer for 30 minutes on 3 consecutive days or in the autoclave at 110° for 15 minutes.

(k) Abel (1912).
(1) Chop 500.0 g. of fat free meat and add to a liter of water at 50°C.
(2) Keep at 50°C. for 30 minutes and then boil for 30 to 45 minutes.
(3) Filter or strain the fluid from the meat.
(4) Make up the fluid to one liter.
(5) Dissolve 1.0% Witte's peptone, 0.5% NaCl and 1.0 to 1.5% gelatin in (4) by heating in the steamer.
(6) Neutralize to litmus.
(7) Steam for 15 to 30 minutes.
(8) Filter and readjust the reaction. If not clear, clarify by the addition of an egg white or meat juice.
(9) Tube or flask.
(10) Sterilize on each of 3 successive days in the steamer or autoclave for 15 minutes at 120°C.

(l) Abel (1912).
(1) Chop 500.0 g. fat free meat and add a liter of water at 50°C.
(2) Keep at 50°C. for 30 minutes and then boil for 30 to 45 minutes.
(3) Filter or strain the fluid from the meat.
(4) Make up the fluid to a liter.
(5) Place 500.0 cc. of (4) in a white enamelled sauce pan and heat to about 70°C.
(6) Add 10.0 g. peptone and 5.0 g. NaCl and stir with a small wooden spoon.
(7) Add the gelatin and stir until dissolved.
(8) Make up to 1 liter by the addition of (4).
(9) Add KOH until the reaction is slightly alkaline to litmus.
(10) Add the beaten white of one egg.
(11) Steam for 30 minutes.
(12) Filter thru two thicknesses of Swedish or Rhenish filter paper moistened with distilled water.
(13) Sterilize in the steamer for 30 minutes on each of 2 successive days.

(m) Forster (Abel 1912).
(1) Chop 500.0 g. of fat free meat and add to a liter of water at 50°C.
(2) Keep at 50°C. for 30 minutes and then boil for 30 to 45 minutes.
(3) Filter or strain the fluid from the meat.
(4) Make up the fluid to one liter.
(5) Dissolve 10.0 g. Peptone (Witte or Chapoteaut) and 5.0 g. NaCl in (4) by heating in the steamer.
(6) Neutralize to litmus.
(7) Steam for 15 to 30 minutes.
(8) Filter and readjust the reaction. Filter until clear.
(9) Tube or flask.
(10) Sterilize on each of 3 successive days in the steamer or autoclave for 15 minutes at 120°C.
(11) Dissolve 100 to 150.0 g. gelatin in (10) by heating at 60°C.
(12) Add KOH until the acidity is faint and then add Na_2CO_3 to make slightly alkaline.
(13) Add the white of an egg.
(14) Place the vessel in a large pot filled with boiling water.
(15) Boil the gelatin for 15 minutes.
(16) Filter thru a sterile hot water funnel at 60°C.
(17) Tube under aseptic conditions.
(18) Heat for 20 minutes in the steamer.
(19) Immerse in cold water.

(n) Löhnis (1913).
(1) Add 1000.0 cc. distilled water to 500.0 g. of finely minced lean beef.
(2) Infuse for 24 hours in the ice chest

or heat for one hour in the water bath at 55°C.
- (3) Boil for 60 minutes in the steamer or in a covered dish.
- (4) Filter thru a clean cloth rising pressure (meat press).
- (5) Cool and remove the fat by filtering thru S. and S. filter paper.
- (6) Make up the volume to 1000.0 cc. by the addition of water.
- (7) Add 1.0% Witte's peptone and 0.5% NaCl.
- (8) Steam for one-half hour.
- (9) Filter.
- (10) Cool.
- (11) Adjust the reaction.
- (12) Steam again for 30 minutes.
- (13) Filter.
- (14) Add 100.0 g. of the best quality of gelatin and soak 2 hours at room temperature.
- (15) Steam 5 minutes.
- (16) Cool.
- (17) Titrate and adjust the reaction.
- (18) Steam 30 minutes.
- (19) Filter thru S. and S. filter paper washed with sterile boiling water.
- (20) Tube.
- (21) Heat on 3 successive days for 15 minutes, 10 minutes and 5 minutes, respectively, at 100°C.

(o) Meier (1918).
- (1) Boil 500.0 g. of fat and tendon free beef in 1 liter of water.
- (2) Filter.
- (3) Dissolve 110.0 g. gelatin, 10.0 g. peptone (Witte) and 5.0 g. NaCl in the filtrate.
- (4) Neutralize by the addition of KOH. Add KOH until turmeric paper is turned quite weakly brownish red.
- (5) Sterilization not specified.

(p) Worth (1919).
- (1) Heat 500.0 g. of chopped beef in 1000.0 cc. water in a water bath at 50 to 55°C. for one hour.
- (2) Strain thru cloth and restore volume.
- (3) Add 10.0 g. peptone, 5.0 g. NaCl and 100.0 g. gelatin to (2).
- (4) Dissolve, filter, adjust to 1.0% acid.
- (5) Sterilize for 20 minutes at 110°C.

(q) Besson (1920).
- (1) Remove all fat and tendons from beef and chop into small pieces.
- (2) Allow 500.0 g. of (1) to macerate with 1000.0 cc. of cold water for 6 hours, or if one wishes to remove the sugar, 12 hours at 37°C.
- (3) Place in an enamelled pot and bring slowly to a boil.
- (4) Boil for 10 minutes.
- (5) Throw on a thick cloth and press the meat free from juice.
- (6) Filter the juice thru moistened paper.
- (7) Make up to 1000.0 cc.
- (8) Add 10.0 g. Chapoteaut's peptone, 5.0 g. NaCl, and 80.0 to 130.0 g. gelatin (extra) (80.0 g. in winter and 130.0 in summer) to (7).
- (9) Heat slowly in an enamelled kettle.
- (10) After solution is complete, boil for 2 or 3 minutes.
- (11) Make slightly alkaline by the addition of soda.
- (12) Heat at 115° for 5 minutes.
- (13) Filter thru paper in the autoclave or use a hot water funnel.
- (14) Tube.
- (15) Sterilize at 110° for 20 minutes.

(r) Abbott (1921).
- (1) Add 500.0 g. of chopped lean beef to 1 liter of water and soak for 24 hours, kept at ice box temperature.
- (2) Strain thru a coarse towel and press until a liter of fluid is obtained.
- (3) Dissolve 10.0 g. (1.0%) peptone, 5.0 g. (0.5%) NaCl and 10.0 to 12.0% gelatin in (2).
- (4) Make slightly alkaline or neutral (indicator not specified).
- (5) Place in a porcelain lined saucepan, and boil over a flame until all the albumin is coagulated and the fluid portion is of a clear pale straw color.
- (6) Filter thru a folded paper.
- (7) Sterilize by steam (method not given).

(s) Dopter and Sacquépée (1921).
- (1) Add 1000.0 cc. of water to 500.0 g.

finely chopped fat and tendon free beef.
- (2) Allow to stand in the ice box for 12 hours, or heat at 50 to 55° for 30 minutes.
- (3) Heat slowly to boiling.
- (4) Boil slowly for 10 minutes stirring constantly.
- (5) Strain thru a cloth.
- (6) Heat slowly (not above 80°C.) and add 20.0 g. peptone, 5.0 g. NaCl and 120.0 g. of gelatin per liter of filtrate.
- (7) Shake until solution is complete.
- (8) Add soda solution to neutralize to litmus.
- (9) Heat in the autoclave at 112° for 10 minutes.
- (10) Filter thru paper.
- (11) Distribute.
- (12) Sterilize at 110° for 10 minutes. (Do not heat over 110°C.)
- (13) Store in a cool place.

(t) Harvey (1921–22).
- (1) Proceed as in the preparation of infusion broth, see variant (bb) of medium 779, step (1) thru (9).
- (2) Add 10.0 g. peptone, 5.0 g. sodium chloride and 120.0 g. gelatin.
- (3) Heat gently with constant stirring.
- (4) Steam or boil 45 minutes to obtain complete solution.
- (5) Bring the volume up to 1000.0 cc. by the addition of hot water.
- (6) Adjust reaction to a definite pH value, or faintly alkaline to litmus or 1.0% acid to phenolphthalein.
- (7) Steam 30 minutes.
- (8) Filter, while hot, thru well-wetted, thick filter paper by placing filter funnel, stand and receptacle for filtrate in the steam sterilizer, and steaming till filtration is complete.
- (9) Distribute into flasks or test tubes.
- (10) Sterilize.
- (11) Cool the flasks or test tubes on final removal from the sterilizer.
- (12) Store in a cool place.

(u) Pitfield (1922).
- (1) Cover 500.0 g. of finely cut fat free beef with 1000.0 cc. water.
- (2) Shake well and place on ice over night.
- (3) Squeeze out the fluid by means of a cloth and make up the volume to 1 liter.
- (4) Inoculate with a culture of the colon bacillus.
- (5) Allow to stand at room temperature over night.
- (6) Boil and add 10.0 g. Witte's peptone and 5.0 g. NaCl.
- (7) Weigh the saucepan and contents and heat to 60°C.
- (8) Make up the loss in weight by the addition of water.
- (9) Neutralize to litmus.

(v) Klimmer (1923).
- (1) Prepare meat infusion.
- (2) Dissolve 10.0 g. peptone, 5.0 g. NaCl and 100.0 g. gelatin in (1) by heating between 50 and 60°C. Do not heat above 60°C.
- (3) Neutralize to litmus.
- (4) Filter.
- (5) Add 5.0 cc. of normal soda solution.
- (6) Sterilize by heating for 40 minutes on each of 3 successive days for 15 minutes in streaming steam.

(w) Park, Williams and Krumwiede (1924).
- (1) Dissolve 100.0 g. gelatin, 10.0 g. peptone and 5.0 g. NaCl in 1000.0 cc. meat juice.
- (2) Coagulate the albumin present in the meat juice to clarify.
- (3) The coagulation of the albumin present in the meat juice clears the medium.
- (4) Filter thru cotton.
- (5) Tube and sterilize in the Arnold sterilizer 20 to 30 minutes on 3 successive days.

(x) Park, Williams and Krumwiede (1924).
- (1) Add "10.0 g. (1.0%) peptone and sodium chloride 0.5 g.," to 1000.0 cc. of meat infusion.
- (2) Heat nearly to boiling and add 100.0 g. gelatin. Dissolve with as little heat as possible.
- (3) Adjust the reaction neutral to litmus (pH = 6.8 to 7.0).
- (4) Cool to 50°C. and clarify by heating for 45 minutes in an Arnold sterilizer.
- (5) Filter.

(6) Tube.

(7) Sterilize in the Arnold 20 to 30 minutes on 3 successive days.

References: Schultz (1891 p. 62), Acosta and Grande (1892 #14), Frothingham (1895 p. 55), Forster (1897 p. 342), Jensen (1898 p. 406), Committee A. P. H. A. (1899 p. 77), Migula (1901 p. 13), Thoinot and Masselin (1902 pp. 30, 32), Frost (1903 p. 6), Abel (1912 p. 15, 17), Löhnis (1913 p. 14), Meier (1918 p. 435), Worth (1919 p. 608), Besson (1920 p. 39), Abbott (1921 p. 125), Dopter and Sacquépée (1921 p. 124), Harvey (1921–22 p. 70), Pitfield (1922 p. 116), Klimmer (1923 p. 192), Park, Williams and Krumwiede (1924 p. 117).

2280a. Koch's Nutrient Gelatin

Constituents:

1.	Any nutrient medium, double strength	1000.0 cc.
2.	Gelatin solution, 5 to 6%	1000.0 cc.

Preparation:

(1) Place gelatin in distilled water and dissolve by warming. A concentration of 5 to 6% gelatin recommended.

(2) Add this gelatin solution to an equal volume of any nutrient medium (double strength).

(3) Neutralize with sodium or potassium hydroxide or phosphate.

(4) Boil and filter off precipitate.

(5) Place in sterilized cotton stoppered flasks.

Sterilization: Sterilize by boiling.

Use: General culture medium and isolation of cholera bacillus.

Variants:

(a) The author stated that larger quantities of gelatin might be used, that the gelatin may be dissolved directly in the nutrient medium.

(b) Hueppe (1885) prepared the medium as follows:

(1) Add 5 or 10% finely cut gelatin to any desired nutrient medium.

(2) Allow to soak for half an hour.

(3) Heat to dissolve.

(4) Neutralize by the addition of sodium carbonate, or make slightly alkaline to litmus.

(5) Heat on the water bath for an hour and filter thru a damp filter paper.

References: Koch (1881 p. 169), Hueppe (1885 p. 101–104), Koch (1887 p. 161).

2281. Smith's Peptone Infusion Gelatin

Constituents:

1.	Distilled water	1000.0 cc.
2.	Lean beef	500.0 g.
3.	Peptone (Witte)	10.0 g.
4.	Gelatin (best white French)	10.0 g.

Preparation:

(1) Prepare a medium from the constituents, method not given.

(2) Add enough KOH to neutralize to litmus.

Sterilization: Sterilize (method not given). After sterilization reaction slightly acid to litmus.

Use: Smith used the medium for the cultivation of *Pseudomonas campestris Pammel.* He reported that the gelatin was slowly but distinctly liquefied. Slight growth on this medium. The presence of salt hindered the growth. Would not grow on a medium alkaline to phenolphthalein. Similar media were used as general culture media and also in water analysis.

Variants:

(a) Committee A. P. H. A. (1901) recommended the following method of preparation:

(1) Infuse 500.0 g. lean meat 24 hours with 1000.0 cc. of distilled water in refrigerator.

(2) Make up loss by evaporation.

(3) Strain infusion thru cotton flannel.

(4) Weigh filtered infusion.

(5) Add 1.0% Witte's peptone and 10.0% gold label sheet gelatin.

(6) Warm on water bath, stirring till peptone and gelatin are dissolved and not allowing the temperature to rise above 60°C.

(7) Heat over boiling water (or steam) bath for 30 minutes.

(8) Restore loss by evaporation.

(9) Titrate after boiling one minute to expel carbonic acid.

(10) Adjust reaction to −1.0% by adding normal hydrochloric acid or sodium hydrate as required.

(11) Boil 2 to 5 minutes over a free flame constantly stirring.

(12) Make up loss by evaporation.

(13) Filter thru absorbent cotton and cotton flannel, passing the filtrate thru the filter until clear.
(14) Titrate and record the final reaction.
(15) Tube, using 5.0 cc. in each tube in the case of gelatin.
(16) Sterilize 15 minutes in the autoclave at 110° or for 30 minutes in streaming steam on three successive days.
(17) Store in the ice chest in a moist atmosphere to prevent evaporation.

(b) Committee A. P. H. A. (1905) prepared the medium as given in variant (a), but sterilized for 5 minutes in the autoclave at 120°C. or for 30 minutes in streaming steam on each of 3 successive days. Cool quickly on ice following heating.

(c) Giltner prepared the medium as follows:
(1) Prepare meat infusion.
(2) Dissolve 1.0% peptone and 15.0% gelatin in a mixture of equal parts (1) and distilled water.
(3) Adjust the reaction to +1.0%.
(4) Sterilization not specified.

References: Smith (1897 p. 480), Committee A. P. H. A. (1901 p. 384), (1905 p. 107), Giltner (1921 p. 380).

2282. Loeffler's Malachite Green Infusion Gelatin

Constituents:

1. Water	5000.0 cc.
2. Beef	4.0 lbs.
3. Gelatin (15.0%)	750.0 g.
4. Peptone (Witte 1.0%)	50.0 g.
5. NaCl (0.5%)	25.0 g.
6. Malachite green solution	
7. Phosphoric acid	

Preparation:
(1) Chop four pounds clean lean beef.
(2) Mix 5 liters tap water with (1).
(3) Add 15.0% = 750.0 g. gelatin, 1.0% = 50.0 g. Witte peptone, 0.5% = 25.0 g. NaCl.
(4) Slowly heat to complete solution of gelatin.
(5) Heat 45 minutes.
(6) Neutralize to litmus with sodium carbonate.
(7) Heat and filter.
(8) To each 100.0 cc. add 3.0 cc. of a normal phosphoric acid and 2.0 cc. of 2.0% malachite green solution.

Sterilization: Not specified.

Use: Isolation of typhoid bacilli from feces.

References: Loeffler (1906 p. 289), Klimmer (1923 p. 213).

2283. Roux and Rochaix's Peptone Infusion Gelatin

Constituents:

1. Distilled water	1000.0 cc.
2. Beef	500.0 g.
3. NaCl	5.0 g.
4. Peptone	10.0 g.
5. Sodium or potassium phosphate	2.0 g.
6. Gelatin (15.0 to 18.0%)	150.0 to 180.0 g.

Preparation:
(1) Chop 500.0 g. of fat and tendon free beef into very small pieces.
(2) Add 1 liter of water and allow to stand over night in the cold.
(3) Press the juice thru a linen cloth by means of a meat press.
(4) Make up the volume to 1000.0 cc. by the addition of water.
(5) Add 5.0 g. NaCl, 2.0 g. of sodium or potassium phosphate and 10.0 g. of peptone.
(6) Boil for an hour.
(7) Filter thru a linen cloth and then thru paper.
(8) Adjust the reaction slightly alkaline to litmus by addition of $KHCO_3$ solution, drop by drop.
(9) Add 15.0 to 18.0% gelatin and heat on the water bath until solution is complete.
(10) Neutralize and make slightly alkaline again to litmus.
(11) Filter using a hot water funnel.
(12) Distribute in flasks or tubes.

Sterilization: Sterilize on 2 or 3 successive days at 100°C.

Use: General culture medium.

Variants:

(a) Choquet's (Besson) prepared a medium as follows:
(1) Dissolve 5.0 g. peptone and 35.0 g. gelatin in 500.0 cc. beef juice.
(2) Heat in the autoclave.

(3) Filter in the autoclave or use a hot water funnel.
(4) Tube.
(5) Add magnesium phosphate $Ca_3(PO_4)_2$ and $CaCO_3$ to each tube. (50.0 g. $Ca_3(PO_4)_2$, 5.0 g. magnesium phosphate and 10.0 g. $CaCO_3$ were used for the entire amount of medium.)
(6) Sterilization not specified.

(b) Besson prepared a similar medium as follows:
(1) Remove all fat and tendons from beef and chop into small pieces.
(2) Allow 500.0 g. of (1) to macerate with 1000.0 cc. of cold water for 6 hours, or if one wishes to remove the sugar, 12 hours at 37°C.
(3) Place in an enamelled pot and bring slowly to a boil.
(4) Boil for 10 minutes.
(5) Throw on a thick cloth and press the meat free from juice.
(6) Filter the juice thru moistened paper.
(7) Make up to 1000.0 cc.
(8) Add 10.0 g. Chapoteaut's peptone, 5.0 g. NaCl, a pinch of sodium phosphate and 80.0 to 130.0 g. gelatin (80.0 g. in winter, 130.0 g. in summer).
(9) Heat slowly in an enamelled kettle.
(10) After solution is complete, boil for 2 or 3 minutes.
(11) Make slightly alkaline by the addition of soda.
(12) Heat at 115°C. for 5 minutes.
(13) Filter thru paper in the autoclave or use a hot water funnel.
(14) Tube.
(15) Sterilize at 110° for 20 minutes.

References: Roux and Rochaix (1911 p. 111), Besson (1920 pp. 38, 41).

2284. Müller's Indicator Infusion Gelatin

Same as medium 784, but solidified by the addition of gelatin.

2285. Jackson and Muer's Liver Infusion Gelatin

Constituents:

1. Water	1000.0 cc.
2. Liver, beef	500.0 g.
3. Gelatin	100.0 g.
4. Peptone (Witte's)	10.0 g.
5. Glucose	10.0 g.
6. Potassium phosphate (K_2HPO_4)	1.0 g.

Preparation:
(1) Chop 500.0 g. beef liver into small pieces and add 1000.0 cc. water Weigh the infusion and container.
(2) Boil blowly for 2 hours in a double boiler, starting cold, and stirring it occasionally.
(3) Make up the loss in weight by evaporation and strain thru a wire strainer.
(4) Cool the filtrate to 50°C. Add 3, and stir a few minutes until dissolved.
(5) Add 5 and 6.
(6) Stir until the ingredients are dissolved, keeping the temperature below 50°C.
(7) After warming this mixture in a double boiler and stirring it for a few minutes to dissolve ingredients, titrate with N/20 sodium hydrate using phenolphthalein as an indicator, and neutralize with normal sodium hydrate.
(8) Boil vigorously for 30 minutes in a double boiler, and 5 minutes over a free flame with constant stirring to prevent the caramelization of the dextrose.
(9) Make up any loss in weight by evaporation and filter thru cotton flannel and filter paper.

Sterilization: Sterilize in an autoclave for 15 minutes at 15 pounds pressure.

Use: Identification of certain bacteria. Used also for cultivation of *B. sporogenes*.

Reference: Jackson and Muer (1911 p. 290), (1911 p. 928).

2286. Fuhrmann's Glucose Infusion Gelatin

Constituents:

1. Horse meat infusion	1000.0 cc.
2. Gelatin	100.0 g.
3. Peptone (Witte)	20.0 g.
4. Dextrose	10.0 g.
5. NaCl	5.0 g.

Preparation:
(1) Prepare horse meat infusion.
(2) Dissolve 2, 3, 4 and 5 in (1).
(3) Pour in plates.

Sterilization: Not specified.

Use: Cultivation of *Pseudomonas cerevisiae.* The author reported that best growth was obtained when the reaction was 1.0% N/1 alkali.

Variants: The author gave the following method of preparation:

(1) Digest 1000.0 g. of horse meat with 1 liter of water for 24 hours in the cold.
(2) Boil for one hour in the steamer.
(3) Cool and filter. Make up to 1 liter.
(4) Add 40.0 g. of Witte's (siccum) peptone, 20.0 g. glucose, and 10.0 g. of NaCl. (This is a stock bouillon.)
(5) To 500.0 cc. of (4) and 100.0 g. gelatin.
(6) Neutralize and clarify.
(7) Dilute with an equal volume of water.
(8) Add N/1 acetic acid or N/1 NaOH to obtained the desired reaction.
(9) Sterilization not specified.

Reference: Fuhrmann (1906 pp. 310, 316).

2287. Choquet's Glycero Phosphate Infusion Gelatin (Besson)

Constituents:

1. Beef juice	300.0 cc.
2. Gelatin (extra white)	35.0 g.
3. Peptone (Chapoteaut)	5.0 g.
4. Glycerol-phosphate of lime	5.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in beef juice.
(2) Heat in the autoclave.
(3) Filter hot in the autoclave or use a hot water funnel.
(4) Tube.

Sterilization: Not specified.

Use: Cultivation of organisms from dental caries.

Reference: Besson (1920 p. 40).

2288. Teague and Clurman's Congo-Red Brilliant Green Glucose Gelatin

Constituents:

1. Infusion gelatin (5.0%)	500.0 cc.
2. Congo red (2.0%)	40.0 cc.
3. Brilliant green	0.415 g.
4. Glucose (0.5%)	2.5 g.
5. Bromoform	

Preparation:

(1) Exact method of preparation of 5.0% nutrient gelatin or composition not given. It is to be prepared however from meat infusion, not from beef extract, and the reaction to be 1.0+.
(2) Prepare a 2.0% solution of congo red.
(3) Add 4.0 cc. of (2) to every 50.0 cc. of (1), also add 0.83% brilliant green and 0.5% glucose.
(4) Add 25.0 cc. of bromoform to 100.0 cc. of sterile distilled water.
(5) Shake thoroly and allow to stand at room temperature over night.
(6) Pipette some of the saturated solution into a sterile flask (do not disturb the bromoform at the bottom of the flask, do not include any of the globules floating on the surface).
(7) One cc. of (6) (saturated bromoform solution) is added to each 10.0 cc. of (3), (congo-red-brilliant-green-gelatin) just before use.

Sterilization: Not specified.

Use: Enrichment medium for typhoid organisms.

Reference: Teague and Clurman (1916–17 p. 125).

2289. Kowalski's Glycerol Lung Infusion Gelatin

Constituents:

1. Water.
2. Lung, calf.
3. NaCl.
4 Potassium phosphate.
5. Na_2SO_4.
6. $(NH_4)_2SO_4$.
7. Sucrose.
8. Peptone.
9. Gelatin (10.0 to 15.0%).
10. Glycerol (8.0 to 10.0%).

Preparation:

(1) Grind one kilogram fresh calf lung.
(2) Add 2 liters water. Boil in glass vessel 30 minutes.
(3) Filter, pressing the meat.
(4) Dissolve 3, 4, 5, 6, 7 and 8 in (3).
(5) Add 10–15.0% gelatin.
(6) Dissolve by heat.
(7) Neutralize with equal parts of sodium and potassium hydrate.
(8) Make to 2.5 liters with distilled water.
(9) Cool to 58°C.
(10) Add the beaten whites of 4 eggs.
(11) Heat a few minutes.
(12) Filter.
(13) Add 8–10.0% glycerol to filtrate.
(14) Distribute in tubes or flasks.

Sterilization: Sterilize intermittently on 3 successive days.

Use: Cultivation of tubercle bacilli.

Variants: Dittrich solidified medium 1737 with gelatin instead of agar.

Reference: Kowalski (1890 p. 266).

2290. Uffelmann's Methyl Violet Infusion Gelatin

Constituents:

1. Infusion gelatin	1000.0 cc.
2. Citric acid	
3. Methyl violet	

Preparation:

(1) Prepare meat infusion peptone gelatin in usual way, making slightly alkaline and fiter.

(2) Add solution of citric acid in such amount that 10.0 cc. of medium requires 14.0 cc. of a solution of 5.3 g. sodium carbonate in 1000.0 cc. water to neutralize exactly.

(3) Filter.

(4) Add to each 100.0 cc. of the filtrate, 2.5 mg. of methyl violet that has been rubbed up with 1 drop absolute alcohol and 1.0 cc. distilled water.

Sterilization: Sterilize in streaming steam 15 minutes.

Use: Isolation of typhoid bacillus. The author reported that the medium eliminated many non-typhoid organisms. Good growth of typhoid bacilli.

Reference: Uffelmann (1891 p. 855).

2291. Vincent's Glucose Glycerol Gelatin

Constituents:

1. Infusion broth	500.0 cc.
2. Gelatin (extra)	50.0 to 75.0 g.
3. Glucose	5.0 g.
4. Glycerol	5.0 g.

Preparation:

(1) Prepare infusion broth from beef and peptone. (Method not given.)

(2) Dissolve 2, 3 and 4 in (1).

(3) Neutralize. (Indicator not specified.)

(4) Tube.

Sterilization: Method not given.

Use: Cultivation of anaerobes in water analysis.

Reference: Vincent (1907 p. 65).

2292. Huntoon's Hormone Gelatin

Constituents:

1. Water	1000.0 cc.
2. Heart, fresh beef (or steak)	500.0 g.
3. Peptone (Bacto)	10.0 g.
4. Gelatin	10.0 g.
5. Salt	5.0 g.
6. Egg, whole	1

Preparation:

(1) Chop 2 and mix 1, 2, 3, 4, 5 and 6. Place in an enamel ware vessel or a large coffee pot.

(2) Heat over a free flame with constant stirring until the red color of the meat infusion changes to brown at a temperature of about 68°C. Do not go beyond this temperature.

(3) Adjust to slightly alkaline to litmus with N/1 NaOH and then add 1.0 cc. per liter of medium.

(4) Cover the vessel and place in an Arnold sterilizer or in a water bath at 100° for 1 hour.

(5) Remove the vessel from the sterilizer and separate with a glass rod, the firm clot which has formed from the side of the vessel.

(6) Return to the Arnold sterilizer at 100° for 90 minutes.

(7) Remove the vessel and allow to stand at room temperature for about 10 minutes in a slightly inclined position.

(8) Pipette off the fluid portion or decant. If it is poured thru a fine wire sieve, many of the fine pieces of meat clot may be caught. (Avoid filtering thru cheese cloth, cotton or other adsorptive materials.)

(9) Allow (8) to stand in tall cylinders for 15 to 20 minutes until the fat present has risen to the surface and removed.

(10) Tube in 10.0 cc. lots.

(11) The medium may be further cleared by filtering thru glass wool, asbestos wool, sedimentation or centrifugation.

Sterilization: Sterilize in the steamer using the intermittent method.

Use: Substitute for media containing serous fluids. The author reported the medium gave as good results as the average grade of serum media.

Reference: Huntoon (1918 p. 171).

2293. Rosenberg's Basal Extract Gelatin

Constituents:

1. Water	1000.0 cc.
2. Gelatin (10.0%)	100.0 g.
3. Peptone (Koch) (4.0%)	40.0 g.
4. Meat extract (0.5%)	5.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Dissolve one of the added nutrients in (1).
(3) Gelatin may be neutralized by the addition of Na_2CO_3 or used without adjusting the reaction.

Sterilization: Not specified.

Use: Bacterial count of water. Used also as a general culture medium.

Added nutrients: Rosenberg added 0.5% of any desired sugar.

Variants:

(a) Same as medium 1693, but using 20.0 g. gelatin instead of 10.0 g. of agar. The agar was used for those organisms liquefying gelatin.
(b) Percival prepared a medium as follows:
  (1) Dissolve 5.0 g. Lemco meat extract and 10.0 g. Witte's peptone in 1000.0 cc. water at ordinary room temperature.
  (2) Add 100.0 g. gelatin (best sheet) and soak for 30 minutes.
  (3) Heat in a water bath until the gelatin is dissolved. Shake from time to time.
  (4) Neutralize to phenolphthalein by the addition of normal caustic soda.
  (5) Add 10.0 cc. of normal HCl to each 1000.0 cc. of the medium.
  (6) Cool the medium to 40°C. and slowly add the white of an egg well beaten up in a little water.
  (7) Heat in the steam sterilizer or water bath.
  (8) Filter the hot solution thru moistened hot filter paper into a sterilized flask. Use a hot water funnel for filtering.
  (9) Tube in 8 to 10.0 cc. quantities.
  (10) Steam for 20 minutes on 3 successive days.
  (11) Add 20.0 g. lactose or glucose.
  (12) To prepare litmus glucose gelatin add 2.0% glucose and enough Kubel-Tiemann neutral litmus to color it a purple tint before sterilization.

References: Rosenberg (1886 p. 452), Banning (1902 p. 426), Percival (1920 p. 47).

2294. Bürger's Basal Fuchsin Sulphite Extract Gelatin

Constituents:

1. Distilled water	900.0 cc.
2. Meat extract (Liebig's)	10.0 g.
3. Peptone (Witte Rostock)	10.0 g.
4. NaCl	5.0 g.
5. Gelatin	100.0 g.
6. Na_2SO_3	2.5 g.
7. Fuchsin, alc. solution	5.0 cc.
8. Soda (crystalline)	1.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1 by heating in the steamer.
(2) Allow (1) to cool.
(3) Add 100.0 g. of gelatin to the nonfiltered nutrient medium.
(4) Allow to soak for an hour or to stand in the ice box over night.
(5) Dissolve the gelatin in the steamer requiring about 30 minutes.
(6) Neutralize with N/1 NaOH to litmus. Shake well after addition of NaOH.
(7) Make alkaline by the addition of 1.0 g. crystalline soda.
(8) Add 10.0 g. of one of the added nutrients either in a solid state or in a concentrated solution.
(9) Cool to about 40–50°C.
(10) Dissolve 1.5 g. of egg albumin in 25.0 cc. of luke warm water, and add to (9). Shake thoroly.
(11) Boil in the steamer for about 30 minutes.
(12) Filter hot, thru sterile paper at a temperature of 50 or 60°C. This may be carried out in the steamer after the flame has been removed. May allow to filter over night.
(13) Distribute in 100.0 cc. lots.
(14) When ready for use, melt sterile (13) and to each 100.0 cc. add 5.0 cc. of a saturated filtered alcoholic solution of fuchsin and 02.5 g. of crystalline

pure sodium sulfite, either in solid or concentrated clear solution.

(15) When material is dissolved mix well.

Sterilization: Sterilize (13) in the steamer for 20 minutes. Sterilize the final medium for 20 minutes in the steamer.

Use: To obtain bacterial counts in water. Author reported that *B. coli* grew best on medium containing lactose. *B. typhosus* and *B. paratyphosus A and B* and Gärtner's enteritidis grew best on mannitol or glucose. Shiga and dysentery bacilli developed best in glucose medium. Cholera vibrio developed best in presence of sucrose. When one of the sugars were fermented by the organisms a red colony developed. The medium should be colorless when ready for use. On plating on a series of media of this type one may determine the kind of organisms present in the water as well as the relative number.

Added nutrients: The author added 10.0 g. of lactose, mannitol or sucrose to (8).

Variants:

(a) Bürger prepared a similar medium using 20.0 g. Liebig's meat extract, 20.0 g. Witte's Rostock's peptone, 10.0 g. NaCl, 200.0 g. gelatin, 2.0 g. crystalline soda, 20.0 g. of the added nutrients, 10.0 cc. of a saturated alcoholic fuchsin solution and 5.0 g. Na_2SO_3 instead of the amounts indicated. Water under investigation may be added to the sterile medium.

(b) Bürger used the following amounts of constituents in preparing a medium in the same manner as above. Distilled water 750.0 cc., Liebig's meat extract 25.0 g., Witte's Rostock peptone, 25.0 g., NaCl, 12.5 g., gelatin 250.0 g., saturated alcoholic fuchsin solution 12.5 cc. Na_2SO_3 6.25 g., crystalline soda 2.5 g., and 25.0 g. of one of the added nutrients. Water under investigation may be added to the sterile medium.

Reference: Bürger (1916–17 p. 478).

2295. Tausz and Peter's Basal Ragit Gelatin

Constituents:

1. Water	1000.0 cc.
2. Ragit powder	22.0 g.
3. Gelatin	100.0 g.

Preparation:

(1) Boil 22.0 g. of ragit powder with 1 liter of water.

(2) Add slowly 100.0 g. gelatin and bring to the boiling point.

(3) The reaction is slightly alkaline.

(4) Filter in the steamer.

Sterilization: Sterilize on 3 successive days for 30 minutes in streaming steam.

Use: Cultivation of *Bacterium aliphaticum, Bact. aliphaticum liquefaciens.*

Added nutrients: The authors added 0.5% glucose, lactose or other nutrients.

Reference: Tausz and Peter (1919 p. 49).

2296. Buchan's Basal Litmus Extract Gelatin

Constituents:

1. Distilled water	850.0 cc.
2. Peptone	20.0 g.
3. Lemco	10.0 g.
4. Gelatin	100.0 g.
5. Litmus	

Preparation:

(1) Dissolve 2, 3, 4 and 10.0 g. of one of the added nutrients in 1.

(2) Add 10.0 cc. of a 5.0% KOH solution.

(3) Tinge with litmus.

Sterilization: Not specified.

Use: To study fermentation.

Added nutrients: The author added 10.0 g. of any desired carbohydrate, alcohol, etc.

Reference: Buchan (1910 p. 107).

2297. Bacto Nutrient Gelatin. (Dehydrated)

Constituents:

1. Distilled water	
2. Beef extract, Bacto	3.0 g.
3. Peptone, Bacto	5.0 g.
4. Gelatin, Bacto	120.0 g.

Preparation:

(1) Dissolve 128.0 g. of Bacto Nutrient Gelatin (dehydrated) in 1000.0 cc. distilled water by warming.

(2) If sterilized at 15 pounds for 20 minutes pH = 6.6±.

Sterilization: Sterilize for 20 minutes at 15 pounds pressure.

Use: General culture medium. The authors reported that the medium conforms to the "Standard Methods" formula.

Reference: Digestive Ferments Co. (1925 p. 10).

2298. Heinemann's Peptone Extract Gelatin

Constituents:

1. Water (tap)........	1000.0 cc.
2. Meat extract........	2.5 g.
3. Peptone............	10.0 g.
4. Gelatin............	100.0 to 120.0 g.

Preparation:

(1) Dissolve 2.5 g. meat extract in 1150.0 cc. of tap water. (Added 150.0 cc. of water to be lost during preparation.)

(2) Heat and when near boiling add 10.0 g. peptone to (1).

(3) When boiling add 10.0 to 12.0% of the best Gold Label gelatin to (2). Add 2 or 3 sheets of gelatin at a time, stirring constantly.

(4) When solution is complete adjust the reaction to alkaline to litmus or neutral to phenolphthalein and then add 0.5% normal HCl.

(5) Cool to 60°C. and stir in the white of one egg dissolved in 30.0 cc. water.

(6) Heat over the flame on a piece of asbestos without stirring. Heat until the egg albumin is completely coagulated and forms a dry film on top.

(7) Add water to make up any loss in weight over 50.0 g.

(8) Filter thru paper or absorbent cotton previously moistened with hot water.

(9) Tube in 10.0 cc. quantities.

Sterilization: Sterilize in the autoclave for 5 minutes at 120°C. or steam for 3 successive days for 20 minutes each day.

Use: General culture medium.

Variants:

(a) Wherry used the following medium to determine effect of reaction and dryness on liquefaction of gelatin by *Cholera spirillum*.

(1) Dissolve 200.0 g. Gold Label gelatin, 10.0 g. Witte's peptone and 30.0 g. Liebig's beef extract in 1000.0 cc. water.

(2) Divide (1) into two 500.0 cc. lots.

(3) To one lot add NaOH so that after sterilization the final reaction is +0.8.

(4) To the other portion add NaOH so that the final reaction after sterilization is +1.0.

(5) Method of sterilization not given.

(b) Bahr reported that Gärtner's bacillus did not liquefy a medium prepared as follows:

(1) Dissolve 200.0 g. gelatin, 10.0 g. peptone, 20.0 g. meat extract (Cibil's) in 1000.0 cc. of water.

(2) Adjust so that 10.0 cc. of the medium will titrate 0.3 with N/10 NaOH using phenolphthalein as an indicator.

(3) Sterilization not specified.

(c) Commiteee A. P. H. A. (1917) (1920) gave the following method of preparation:

(1) Add 3.0 g. of beef extract and 5.0 g. peptone to 1000.0 cc. of distilled water and add 100.0 g. gelatin, dried for 30 minutes at 105°C. before weighing.

(2) Heat slowly on a steam bath to 65°C. until all the gelatin is dissolved. (May be soaked for 30 minutes before heating.)

(3) Make up lost weight, titrate and if the reaction is not already between +0.5 and +1.0, adjust to +1.

(4) Filter thru cloth and cotton until clear.

(5) Distribute in tubes in 10.0 cc. quantities or in larger amounts as desired.

(6) Sterilize in the autoclave at 15 pounds (120°C.) for 15 minutes after the pressure reaches 15 pounds.

(d) Committee S. A. B. (1918) recommended the same medium as Committee A. P. H. A. (1917) but clarified with egg and adjusted to pH between 6.6 and 7.4.

(e) Treece dissolved 12% gelatin, 2.0% peptone and 0.5% meat extract in water. The medium was used to determine fecal and non-fecal strains of the colon-aerogenes group. The author reported that this medium correlated the fermentation of adonitol. Positive results were indicated by a line of 4 to 8 bubbles extending down the line of isolation.

(f) Committee A. P. H. A. (1923) adjusted the medium to a faint pink with phenol red or to the required tint with brom thymol blue instead of titrating to +1.0% as in 1917.

(g) Committee A. P. H. A. (1925) prepared the medium as follows:
- (1) Add 3.0 g. of beef extract, 5.0 g. peptone and 120.0 g. gelatin (undried market product as stored in the ordinary cupboard) to 1000.0 cc. distilled water.
- (2) Heat slowly on a steam bath to 65°C. until the ingredients are dissolved.
- (3) Make up the lost weight with distilled water and adjust the reaction so that after the final sterilization the pH value will be between 6.2 and 7.0.
- (4) Bring to a boil, stirring vigorously. Make up the lost weight with distilled water and clarify.
- (5) Distribute in the desired containers.
- (6) Sterilize in the autoclave at 15 pounds (120°C.) for 15 minutes, after the pressure has reached 15 pounds.

References: Heinemann (1905 p. 23), Wherry (1905 p. 318), Bahr (1916–17 p. 21), Committee A. P. H. A. (1917 p. 96), Committee S. A. B. (1918 p. 115), Ball (1919 p. 77), Tanner (1919 p. 55), Committee A. P. H. A. (1920 p. 95), Levine (1921 p. 109), Treece (1920 p. 9), Committee A. P. H. A. (1923 p. 95), Park, Williams and Krumwiede (1924 p. 131), Committee A. P. H. A. (1925 p. 97).

2299. Frost's Peptone Extract Gelatin

Constituents:

1. Distilled water	1000.0 cc.
2. Beef extract, Liebig's	3.0 g.
3. Peptone (Witte) (1.0%)	10.0 g.
4. NaCl (0.5%)	5.0 g.
5. Gelatin (Gold Label sheet) (10.0 or 15.0%)	100.0 or 150.0 g.

Preparation:
- (1) Weigh out 3.0 g. of beef extract such as Liebig's.
- (2) Add a liter of distilled water.
- (3) Place in a cooking vessel.
- (4) Add 1.0% peptone (Witte) 0.5% NaCl and from 10.0 to 15.0% the best gold label sheet gelatin. (Add 10.0% gelatin in winter and 15.0% in summer.)
- (5) Weigh the solution and the vessel.
- (6) Heat until solution is complete.
- (7) Neutralize the phenolphthalein.
- (8) Boil 5 minutes and restore the weight lost by the addition of distilled water.
- (9) Test the reaction and readjust if necessary.
- (10) Cool below 60°C. and thoroly stir in an egg.
- (11) Boil until the albumin coagulates and floats in the clear fluid.
- (12) Filter thru cotton supported on a coil of wire using a suction pump to hasten filtration.
- (13) Add 5.0 cc. (0.5%) of a normal HCl solution. In water analysis a +1.0 reaction is used and +0.5 for pathogenic bacteria.
- (14) Tube.

Sterilization: Sterilize in the steamer for 30 minutes on 3 consecutive days or in the autoclave at 110° for 15 minutes.

Use: General culture medium.

Variants:

(a) Stoklasa isolated radiobacter and *Azotobacter chroococcum* on a medium containing 100.0 g. gelatin, 20.0 g. peptone, 5.0 g. NaCl and 5.0 g. Liebig's meat extract per liter of water.

(b) Day and Baker cultivated bacteria causing ropiness in beer on a medium adjusted to +1.0, containing 0.5% Lemco meat extract, 0.5% NaCl, 1.0% Witte's peptone and 12.0% gelatin.

(c) Abel prepared the medium as follows:
- (1) Dissolve 10.0 g. Lemco meat extract, 10.0 g. Witte's peptone and 5.0 g. NaCl in 1000.0 cc. water.
- (2) Steam for 30 minutes.
- (3) Filter when cool.
- (4) Add 900 parts (3) to 100 parts gelatin and allow to soak and soften.
- (5) Place in a steamer until solution is complete (not longer than 30 minutes in the steamer).
- (6) Neutralize to litmus.
- (7) Heat for 15 minutes.
- (8) Readjust the reaction if necessary.
- (9) Add 1.5 parts of crystalline undecomposed soda.

(10) Steam for 30 to 45 minutes, and filter.
(11) Tube in sterile tubes.
(12) Steam once for 15 to 20 minutes.

(d) Bürger made bacterial counts of water on a medium prepared as follows:
(1) Dissolve 10.0 g. Liebig's meat extract, 10.0 g. Witte's Rostock peptone and 5.0 g. NaCl in 900.0 cc. distilled water by heating in the steamer (requires about one hour).
(2) Allow (1) to cool.
(3) Add 100.0 g. gelatin to the nonfiltered nutrient medium.
(4) Allow to soak for an hour or to stand in the ice box over night.
(5) Neutralize with N/1 NaOH to litmus. Shake well after each addition of NaOH.
(6) Dissolve the gelatin in the steamer requiring about 30 minutes.
(7) Make alkaline by the addition of 1.0 g. crystalline soda.
(8) Cool to about 40 or 50°C.
(9) Dissolve 1.5 g. of egg albumin in 25.0 cc. of luke warm water and add to (8). Shake thoroly.
(10) Boil in the steamer for about 30 minutes.
(11) Filter hot, thru sterile paper at room temperature of 50 or 60°C. This may be carried out in the steamer after the flame has been removed. May allow to filter over night.
(12) Distribute in sterile flasks in 100.0 cc. lots or in 10.0 cc. lots in sterile tubes under aseptic conditions.
(13) Sterilize in the steamer for about 20 minutes and cool quickly in cold water.

(e) Bürger also prepared media in the same manner as indicated above using the following amounts of constituents. Using these media, the water under investigation may be added directly to the sterile gelatin.

1. Distilled water..	800.0 cc.	750.0 cc.
2. Meat extract (Liebig's).......	20.0 g.	25.0 g.
3. Peptone (Witte Rostock)........	20.0 g.	25.0 g.
4. NaCl...........	10.0 g.	12.5 g.
5. Gelatin...	200.0 g.	250.0 g.
6. Soda (see step (7) above)......	2.0 g.	2.5 g.

(f) Meier used the following medium for bacterial counts of milk:
(1) Dissolve 125.0 g. gelatin, 10.0 g. Liebig's meat extract, 10.0 g. Witte's (siccum) peptone, and 5.0 g. NaCl in 1000.0 cc. water.
(2) Neutralize to litmus and then add 10.0 cc. N/1 soda solution per liter medium.
(3) Sterilization not specified.

(g) Besson prepared the medium as follows:
(1) Dissolve 5.0 g. Liebig's meat extract in 1000.0 cc. of water.
(2) Dissolve 100.0 g. gelatin in (1).
(3) Boil for 3 minutes.
(4) Neutralize.
(5) Heat at 115° for 5 minutes.
(6) Filter thru paper, in the autoclave or use a hot water funnel.
(7) Tube.
(8) Sterilize at 110° for 20 minutes.

(h) Wolf and Shunk reported that a greater pH range may be obtained by adjustment of reaction after sterilization before solidification using a medium prepared as follows:
(1) Dissolve 100.0 to 150.0 g. gelatin, 3.0 g. Liebig's beef extract, 10.0 g. Peptone (Armour's) and 5.0 g. NaCl in 1000.0 cc. of water by heating in autoclave.
(2) Flask.
(3) Sterilize at 10 pounds for 15 minutes.
(4) Cool to about 40°C.
(5) With sterile pipette, pipette 10.0 cc. quantities into sterile test tubes and add appropriate quantities of strong acid or alkali to give the desired reaction. (Authors used HCl sp. gr. 1.20 or 39.11% and NaOH, sp. gr. 1.226 or approximately 20.0%.)
(6) Mix the tube thoroly and cool. Do not sterilize.

(i) Giltner prepared the medium as follows:
(1) Add 150.0 g. gelatin to a liter of tap water and heat on a water bath until solution is complete.
(2) Cool down to 60°C.

(3) Mix 10.0 g. of egg albumin in 100.0 cc. of water and add to (2).
(4) Add 3.0 g. meat extract, 10.0 g. peptone and 5.0 g. NaCl to (3).
(5) Cover the container, place in the autoclave and heat at about 15 pounds pressure for 45 minutes.
(6) Adjust the reaction to +1.5% by the addition of NaOH or HCl.
(7) Filter while boiling hot thru plaited filter paper, washed with 500.0 g. boiling water.
(8) Distribute as desired.
(9) Autoclave for 20 minutes at 10 pounds pressure.
(10) Cool in a running water bath immediately.

(j) Stitt prepared the medium as follows:
(1) Place 3.0 g. Liebig's extract, 10.0 g. peptone and 5.0 g. NaCl in a mortar.
(2) Dissolve the white of one or two eggs in 1000.0 cc. of water.
(3) Add (2), little by little, to (1), until a brownish color is obtained.
(4) Heat (3) to 45° in a water bath.
(5) Dissolve about 120.0 g. of Gold Label or other good quality gelatin in (4).
(6) Add sufficient normal NaOH to bring the reaction to +1.
(7) Place in the inner compartment of a rice cooker.
(8) Boil for 15 minutes.
(9) Filter thru filter paper in a hot funnel. The paper must be very thoroly wetted with very hot water.
(10) Tube.
(11) Sterilize either in the Arnold on 3 successive days or in the autoclave at 8 to 10 pounds pressure for 10 minutes.
(12) Cool the tubes as quickly as possible.

(k) Klimmer gave the following method of preparation:
(1) Dissolve 10.0 g. Liebig's meat extract, 10.0 g. Witte's peptone, and 5.0 g. NaCl in 1000.0 cc. water by heating for 30 minutes in the steamer.
(2) Allow to stand until cool.
(3) Filter.
(4) To 900.0 g. of (2) add 100.0 g. of gelatin.
(5) Steam until solution is complete (not longer than 30 minutes).
(6) Add 4.0% NaOH solution until blue litmus paper is no longer turned red.
(7) Boil for 15 minutes in the steamer.
(8) Add 1.5 g. of crystalline (not effloresced) soda per liter.
(9) Boil 30 minutes.
(10) Filter.
(11) Tube in 10.0 cc. quantities.
(12) Sterilize by a single heating at 15 to 20 minutes in steam.

References: Frost (1903 p. 6), Stoklasa (1908 p. 489), Abel (1912 p. 19), Day and Walker (1912–13 p. 435), Bürger (1916–17 p. 477), Meier (1918 p. 435), Besson (1920 p. 40), Wolf and Shunk (1921 p. 325) Giltner (1921 p. 35), Stitt (1923 p. 38), Klimmer (1923 p. 193), Park, Williams and Krumwiede (1924 p. 177).

2300. Kohn's Peptone Extract Gelatin

Constituents:

1. Distilled water.....	1000.0 cc.
2. Meat extract (Liebig's)..........	6.0 g.
3. Peptone (Witte's)..	10.0 g.
4. NaCl...............	5.0 g.
5. K_2HPO_4...........	2.0 g.
6. Gelatin.............	100.0 or 120.0 g.

Preparation:
(1) Dissolve 2, 3, 4 and 5 in 1 by heating on the water bath.
(2) Add and dissolve 100.0 to 120.0 g. gelatin in (1).
(3) Neutralize to litmus with NaOH and then add 1.5 g. crystalline soda (15.0 cc. of a 10.0% soda solution).

Sterilization: Not specified.

Use: Medium used for water analysis.

Reference: Kohn (1909 p. 127).

2301. Buchan's Neutral Red Peptone Extract Gelatin

Constituents:

1. Distilled water..............	850.0 cc.
2. KOH (5.0% solution)........	10.0 cc.
3. Gelatin......................	100.0 g.
4. Lemco......................	10.0 g.
5. Peptone.....................	20.0 g.
6. Neutral red	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Add neutral red to give a brilliant color to (1).

Sterilization: Not specified.

Use: To determine fluorescence production by organisms found in ice cream.

Reference: Buchan (1910 p. 108).

2302. von Caron's Nitrate Extract Gelatin

Constituents:

1. Water	1000.0 cc.
2. Peptone (1.0%)	10.0 g.
3. Beef extract (Liebig's) (1.0%)	10.0 g.
4. KNO_3	2.0 g.
5. Gelatin (5.0 to 10.0%)	50.0 to 100.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1.

Sterilization: Not specified.

Use: Isolation of denitrifying bacteria. *Bac. Stutzeri*, *Bac. filefaciens*, *Bact. denitrificans*, *Vibrio denitrificans*, *Bact. nitrovorum*, *Bact. centropunctatum.*

Variants: Percival dissolved 0.5% Lemco or other meat extract, 1.0% peptone, 0.0 or 0.5% NaCl, 0.3 g. $NaNO_3$ and 10.0% gelatin in water. The medium was neutralized by the addition of Na_2CO_3 and sterilizer on 3 successive days for 20 minutes.

References: von Caron (1912 p. 70), Percival (1920 p. 165).

2303. Henneberg's Glucose Extract Gelatin

Constituents:

1. Water	1000.0 cc.
2. Dextrose	20.0 to 50.0 g.
3. Meat extract	10.0 g.
4. Peptone	10.0 g.
5. NaCl	2.0 g.
6. Gelatin	50.0 to 100.0 g.

Preparation: (1) Dissolve 2, 3, 4, 5 and 6 in 1.

Sterilization: Not specified.

Use: Cultivation of acetic acid bacteria *B. Pasteurianum*, *B. oxydans*, *B. aceti*, *B. Kützingianum.* Banning used a similar medium to study oxalic acid formation by *Bact. industrium* Henneberg, *Bact. oxydans* Henneberg, *Thermobacterium aceti* Zeidler, *Bact. acidi oxalic*, *Bact. monasteriense*, *Bact. diabeticum*, *Bact. dortmundense*, and *Bact. parvulum.* Agar was used to grow those organisms that liquefy gelatin. Oxalic acid was formed by these organisms. Wash a culture from the surface of the agar or gelatin with H_2SO_4. Filter and evaporate. Oxalic acid crystals will form.

Variants: Banning prepared a medium by dissolving 20.0 g. glucose, 10.0 g. peptone, 10.0 g. meat extract and 70.0 g. gelatin in 1000.0 cc. water. The reaction was slightly acid.

References: Henneberg (1898 p. 18), Banning (1902 p. 397, 426).

2304. Gottheil's Glucose Extract Gelatin

Constituents:

1. Water	1000.0 cc.
2. Gelatin	100.0 g.
3. Glucose	10.0 g.
4. NaCl	2.0 g.
5. Meat extract (Liebig's)	8.0 g.
6. Peptone (Witte)	12.0 g.

Preparation:

(1) Dissolve 4, 5 and 6 in 500.0 cc. of water.

(2) Neutralize by the addition of a concentrated Na_2CO_3 solution.

(3) Heat for a time in a sterilizer.

(4) Filter.

(5) Soak 100.0 g. gelatin in 500.0 cc. of water for about 3 hours.

(6) Add (4) to (5).

(7) Allow to stand for 3 hours at 100°C.

(8) Neutralize once more with Na_2CO_3 and heat for a short time.

(9) Filter.

(10) Add 10.0 g. glucose.

Sterilization: Sterilize on 3 successive days in the steamer.

Use: Gottheil cultivated organisms found in the soil and in the root and rhizomes of leguminous plants. Bachman cultivated obligate anaerobes on a similar medium.

Variants: Bachman dissolved 16.0% gelatin, 1.0% glucose, 1.0% Witte's peptone, 0.8% meat extract, and 0.2% NaCl in water. The reaction was adjusted to a slight alkalinity by the addition of Na_2CO_3.

References: Gottheil (1901 p. 432), Bachman (1912–13 p. 7).

2305. Buchner's Sucrose Extract Gelatin

Constituents:

1. Water	1000.0 cc.

2. Gelatin	100.0 g.
3. Liebig's meat extract	5.0 g.
4. Peptone (puriss)	5.0 g.
5. Sucrose	20.0 g.
6. Na_2HPO_4	5.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.

(2) Make slightly alkaline by the addition of soda.

Sterilization: Not specified.

Use: Cultivation of cholera bacillus.

Variants: Besson used 5.0 g. $Ca_3(PO_4)_2$ instead of Na_2HPO_4.

References: Buchner (1885 p. 368), Weisser (1886 p. 325), Besson (1920 p. 40).

2306. Percival's Urea Extract Gelatin

Constituents:

1. Water	1000.0 cc.
2. Beef extract (Lemco)	5.0 g.
3. Peptone (Witte)	10.0 g.
4. Gelatin	100.0 g.
5. Urea	20.0 g.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) If acid neutralize with $(NH_4)_2CO_3$.

(3) Tube.

Sterilization: Method not given.

Use: Cultivation of urea splitting organisms, *Urobacillus Pasteuri*. The author reported that urea fermenting colonies were surrounded with small white crystals of calcium carbonate.

Reference: Percival (1920 p. 224).

2307. Zipfel's Basal Nutrient Gelatin

Constituent:

1. Nutrient gelatin	1000.0 cc.

Preparation: (1) Prepare nutrient gelatin and dissolve one of the added nutrients.

Sterilization: Not specified.

Use: Zipfel cultivated nodule bacteria.

Added nutrients: The author added one of the following:

Leucine	2.0 or 3.0%
Tyrosine	0.05, 0.1 or 0.2%
Cholesterin	0.02, 0.3 or 0.5%
Asparagin	2.0 or 5.0%
Nucleic acid	0.2, 0.5, 1.0 or 2.0%
Caffeine	0.1, 0.2, 0.3, 0.4 or 0.5%

Variants: Besson added 2.0 to 4.0% of any desired carbohydrate, alcohol, etc., to nutrient gelatin. Just before use the medium was melted and sufficient quantity of litmus added to give a light blue color.

References: Zipfel (1911–12 p. 129), Besson (1920 p. 59).

2308. Wurtz's Nutrient Gelatin

Constituents:

1. Bouillon	1000.0 cc.
2. Gelatin	70.0 g.

Preparation:

(1) Add 70.0 g. gelatin to a liter of bouillon.

(2) Heat on a salt water bath until the gelatin is dissolved.

(3) Adjust the reaction to a slight alkalinity.

(4) Add the whites of 2 eggs beaten up in some water, when the temperature of the gelatin reaches 50°C.

(5) Heat in the autoclave at 115° for one minute.

(6) Filter thru Chardin paper.

(7) Distribute in tubes.

Sterilization: Not specified.

Use: General culture medium.

Variants:

(a) Smith dissolved 10.0 to 15.0% of the best grade of sheet gelatin in bouillon.

(b) Sergent cultivated *Bacterium zopfii* on one of the following combinations:

Bouillon	1000.0 cc.	1000.0 cc.	1000.0 cc.
NaCl	5.0 g.	5.0 g.	
Peptone	10.0 g.	10.0 g.	10.0 g.
Gelatin	130.0 g.	80.0 g.	180.0 g.

(c) Löhnis prepared the medium as follows:

  (1) Add 10.0% of the best Gold Label gelatin to 350 cc. hot bouillon.

  (2) When solution is complete neutralize to litmus.

  (3) Steam for 45 minutes.

  (4) Readjust the reaction if necessary.

  (5) Beat the white of an egg thoroly.

  (6) Add (5) to (4) cooled to about 50°C.

  (7) Heat for 30 to 45 minutes in the steamer.

  (8) Filter thru folded filter paper using a hot water funnel.

  (9) Distribute as desired.

  (10) Steam for 10 to 15 minutes.

(d) Roddy gave the following method of preparation:

  (1) Break up 100 to 300.0 g. of the best

French gelatin in 1000.0 cc. of bouillon.
  - (2) Allow to soak for 5 minutes.
  - (3) Heat.
  - (4) Stir until solution is complete.
  - (5) Sterilize in the steamer for 15 minutes on each of 3 successive days.
- (e) Bezançon prepared the medium as follows:
  - (1) Dissolve 100.0 g. of gelatin (126.0 to 150.0 g. in summer) in 1000.0 cc. bouillon.
  - (2) Cool to 50°C.
  - (3) Add the beaten white of an egg.
  - (4) Adjust the reaction to slightly alkaline.
  - (5) Heat for 15 minutes at 110°C.
  - (6) Filter while hot.
  - (7) Tube.
  - (8) Sterilize at 110°C. for 15 minutes.
- (f) Giltner added 3.0% NaCl to nutrient gelatin and adjusted to −2.0%. The medium was used for the cultivation of phosphorescent halophilic organisms.
- (g) Cunningham prepared the medium as follows:
  - (1) Add 10.0% Coignet's gelatin to bouillon.
  - (2) Steam for 1 hour to dissolve the gelatin.
  - (3) Adjust to a slight alkalinity using turmeric paper as an indicator (distinctly brown).
  - (4) Steam again for 45 minutes.
  - (5) Readjust the reaction if necessary.
  - (6) Cool the medium to 50°C. and add 1.0% egg albumin mixed to a thin paste in a few cc. of water.
  - (7) Steam for 45 minutes.
  - (8) Filter hot thru gray filter paper that has had boiling water poured thru it.
  - (9) Tube in 8.0 cc. quantities.
  - (10) Sterilize intermittently in steam.

References: Wurtz (1897 p. 28), Smith (1902 p. 86), Sergent (1906 p. 1015), Löhnis (1913 p. 15), Roddy (1917 p. 43), Worth (1919 p. 604), Bezançon (1920 p. 113), Giltner (1921 p. 370, Cunningham (1924 p. 13).

2309. Heller's Indicator Nutrient Gelatin

Constituents:

1. Nutrient gelatin.
2. Neutral red (Sat. Aq. soln. Grübler's or other indicator).

Preparation: (1) Add 4 drops of sterile saturated watery solution of Grübler's neutral red to 10.0 cc. of sterile melted gelatin at 40°C.

Sterilization: Method of sterilization of gelatin or neutral red solution not given.

Use: Neutral red test for coli organisms. Author reported that *B. coli* caused a decolorization and fluorescence of this medium after 6 hours. Other investigators used similar media for purposes as indicated.

Variants:

- (a) Calandra added 4 drops of a sterile 1.0% watery solution of brilliant cresyl blue to 10.0 cc. of sterile nutrient gelatin. The gelatin was colored bright green. The medium was used to differentiate colon bacilli from typhoid bacilli. He reported that *B. coli* did not cause decolorization after 24 hours. Typhoid in contrast gave a light green layer on the upper surface. After several days, however, the gelatin was completely decolorized if *B. coli* be present: A thin light green layer remained if typhoid bacilli be present.
- (b) Signorelli added 1.0 cc. of a 1.0% solution of dahlia to each 10.0 cc. of neutral nutrient gelatin. He used the medium to show adsorption of the dye by the cholera vibrio and reported that liquefaction and decolorization took place at the same time. The bacteria were highly colored.
- (c) Signorelli added 1.0 cc. of a 1.0% solution of erythrosin and 1.0 cc. of a 1.0% solution of safranin to 10.0 cc. of neutral nutrient gelatin. He reported that the cholera vibrio adsorbed the dye, being highly colored and that the gelatin was liquefied.
- (d) Besson prepared a medium using Noeggerath's indicator solution.
  - (1) Prepare saturated watery solution of methylene blue, gentian violet, methyl green, chrysoidine and fuchsin.
  - (2) Mix 2.0 cc. of methylene blue 4.0 cc. gentian violet, 1.0 cc. of

methyl green, 4.0 cc. of chrysoidine and 3.0 cc. of fuchsin.
(3) Add 200.0 cc. of distilled water to (2) and allow to stand several hours. The color should be greenish blue. If not obtain the original color by adding either blue, green or red dye.
(4) Sterilize at 100°C.
(5) Add 7 to 10 drops of (4) to a tube of sterile melted nutrient gelatin.

References: Heller (1905 p. 118), Calandra (1910 p. 570), Signorelli (1912 p. 472), Besson (1920 p. 60).

2310. Matzüschita's Glucose Gelatin

Constituents:

1. Bouillon	1000.0 cc.
2. Gelatin	100.0 g.
3. Peptone	10.0 g.
4. NaCl	5.0 g.
5. Glucose	20.0 g.

Preparation: (1) Dissolve 2, 3, 4 and 5 in 1000.0 cc. bouillon.

Sterilization: Not specified.

Use: Cultivation of mammalian and chicken tubercle bacilli. The author reported that the Mammalian and chicken types gave same kind of growth. A white precipitate was formed in the liquid gelatin (at 30°C.). Other investigators used similar media for purposes as indicated below.

Variants:
(a) Rivas solidified medium 923 by the addition of gelatin. This medium was used to cultivate anaerobes.
(b) Frost added 1.0% glucose to nutrient gelatin.
(c) Worth cultivated members of the colon-typhoid group on nutrient gelatin of a reaction 0.4% or 1.0% acid to which was added 2.0% glucose. He also prepared a medium adding calcium carbonate.

References: Matzuschita (1899 p. 128), Rivas (1902 p. 836), Frost (1903 p. 64), Worth (1919 p. 604).

2311. Ramond's Rubine Acid Lactose Gelatin

Same as medium 1796 but solidified by the addition of gelatin instead of agar.

2312. Wurtz's Litmus Lactose Gelatin

Constituents:

1. Nutrient gelatin	1000.0 cc.
2. Lactose (2.0%)	20.0 g.
3. Litmus	

Preparation:
(1) Add 20.0 g. lactose to sterile neutral nutrient gelatin.
(2) Tube.
(3) Add sufficient tincture of litmus to each tube to give a violet color.
(4) Pour sterile (3) into plates.

Sterilization: Sterilize (3) at 100°C.

Use: Differentiation of *Bact. coli*. The author reported that *Bact. coli* fermented lactose with the production of acid. Typhoid bacilli colonies were colorless, colon colonies red.

Variants:
(a) Heinemann gave the following method of preparation:
  (1) Dissolve 1.0% lactose in ordinary 10 or 12.0% gelatin.
  (2) Distribute in 8.0 cc. quantities in tubes.
  (3) Add 1.0% sterile litmus solution to each tube before using (amount of solution not specified).
(b) Abbott prepared the medium as follows:
  (1) Prepare nutrient gelatin so the alkalinity is such that it requires 0.1 cc. of a 1:20 normal H_2SO_4 solution to neutralize 1.0 cc. of medium. Indicator not specified.
  (2) Add 2.0 to 3.0% lactose.
  (3) Decant into test tubes.
  (4) Sterilize in the usual way (method not given).
  (5) Add sufficient sterile litmus tincture to each tube to give a decided but not intense blue color. Add the litmus under aseptic conditions.

References: Wurtz (1897 p. 43), Heinemann (1905 p. 127), Tanner (1919 p. 55), Abbott (1921 p. 142).

2313. Wurtz's Glycerol Gelatin

Constituents:

1. Nutrient gelatin	1000.0 cc.
2. Glycerol (6.0%)	60.0 g.

Preparation: (1) Add 6.0% sterile glycerol to sterile nutrient gelatin under aseptic conditions.

Sterilization: Method not given.
Use: Cultivation of tubercle bacilli.
Reference: Wurtz (1897 p. 46).

2314. Holz's Phenol Gelatin

Constituents:
1. Nutrient gelatin.
2. Phenol (5.0% solution).

Preparation:
(1) Prepare nutrient gelatin in the usual manner.
(2) Neutralize with 10.0% Na_2CO_3 solution, using litmus as an indicator.
(3) Distribute sterile (2) in 10.0 cc. lots by means of a burette.
(4) Heat once more.
(5) Add from 7 to 12 drops of 5.0% phenol solution to each tube. (3 drops = 0.1 cc.)
(6) Pour in plates, or prepare roll cultures.

Sterilization: Sterilize (2) by repeated heatings.
Use: Isolation of typhoid bacilli.
Variants: Migula added 0.5 cc. of a 4.0% phenol solution to each tube (10.0 cc.) of slightly alkaline nutrient gelatin.
References: Holz (1890 p. 144), Migula (1901 p. 24).

2315. Stutzer and Hartleb's Urea Gelatin

Constituents:

1. Nutrient gelatin	1000.0 cc.
2. Urea (2.0%)	20.0 g.

Preparation:
(1) Add 2.0% urea to ordinary nutrient gelatin.

Sterilization: Method not given.
Use: Attempt to cultivate the bacterium of foot and mouth disease. Other investigators cultivated soil bacteria and urea splitters on similar media.
Variants:
(a) Hoffman added 1.0% urea to 10.0% alkaline gelatin. The medium was used for differential count of bacteria from soil. He reported that if urea was fermented the colonies were surrounded by a halo of characteristic biscuit shaped crystals.
(b) Löhnis studied urea decomposition on a medium prepared by adding 1.0 cc. of a 15.0% aqueous solution of urea to tubes of gelatin. This medium was then heated in the steam sterilizer.
(c) Geilinger cultivated urea splitting organisms on nutrient gelatin containing 1.0% urea.
(d) Cunningham determined the decomposition of urea on nutrient 12.0% gelatin containing 2.0% urea. The medium was sterilized by the intermittent method in the steamer.

References: Stutzer and Hartleb (1897 p. 403), Hoffman (1912 p. 387), Löhnis (1913 p. 95), Geilinger (1917 p. 246), Cunningham (1924 p. 143).

2316. Park, Williams and Krumwiede's Meat Gelatin

Constituents:
1. Nutrient gelatin.
2. Meat.

Preparation:
(1) Drop pieces of meat into tubes of nutrient gelatin.

Sterilization: Method not given.
Use: General culture medium.
Reference: Park, Williams and Krumwiede (1924 p. 125).

2317. Worth's Glucose Liver Gelatin

Constituents:

1. Nutrient gelatin	1000.0 cc.
2. Glucose (2.0%)	20.0 g.
3. Liver, rabbit	

Preparation:
(1) Prepare nutrient gelatin and add 2.0% glucose.
(2) Adjust the reaction to +1.0% acid.
(3) Add rabbit liver.

Sterilization: Not specified.
Use: Preservation of cultures.
Reference: Worth (1919 p. 604).

2318. Nastinkoff's Egg Yolk Nutrient Gelatin

Same as medium 1950 but solidified with gelatin instead of agar.

2319. Pergola's Alkaline Blood Gelatin

Constituents:

1. Nutrient gelatin	700.0 cc.
2. Blood, beef	150.0 cc.
3. KOH, normal	150.0 cc.

Preparation:
(1) Collect beef blood in a sterile flask

containing glass beads. Shake to defibrinate the blood.

(2) Mix an equal volume of (1) and N/1 KOH solution.

(3) Prepare nutrient gelatin. It may be necessary to increase the gelatin content of the medium to 20 or 30.0% in order to obtain a solid medium after the addition of the alkaline blood.

(4) Neutralize (3) to litmus.

(5) To 700.0 cc. of the melted neutral nutrient gelatin add 30.0 cc. of sterile (2) and mix.

(6) Distribute into sterile tubes or plates.

Sterilization: Sterilize (2) by heating in the Koch steamer for 30 minutes. Sterilization of gelatin not specified.

Use: Enrichment and detection of cholera vibrio. The author reported that the cholera vibrio liquefied the gelatin.

Reference: Pergola (1910 p. 493).

2320. Müller's Blood Gelatin

Constituents:

1. Nutrient gelatin	1000.0 cc.
2. Blood, goat	100.0 cc.

Preparation:

(1) Distribute nutrient gelatin in 240.0 cc. lots in sterile 400.0 cc. Erlenmeyer flasks.

(2) Allow about 25.0 cc. of blood to pass directly from the juglar vein of a goat into melted gelatin, cooled to about 45°C.

(3) Mix well and pour into sterile Petri dishes.

Sterilization: Method not specified.

Use: Cultivation of fowl diphtheria bacilli. Ito and Matsuzaki cultivated *Spirochaeta icterohaemorrhagiae* on a similar medium.

Variants: Ito and Matsuzaki prepared their medium as follows:

(1) Prepare nutrient gelatin.

(2) Melt (1) and cool to 25–30°C.

(3) Add from 2 to 4 parts of guinea pig blood or human blood to one part of (2). The erythrocytes settle to the bottom of the gelatin before solidification giving it an opaque and deeper color.

(4) Inoculate with a drop of infected blood and mix well by stirring.

(5) May be covered with a layer of paraffin, but not necessary in order to obtain growth. Incubate at 15 to 37°C.

References: Müller (1906 p. 519), Ito and Matsuzaki (1916 p. 558).

2321. Müller's Serum Gelatin

Constituents:

1. Nutrient gelatin	1000.0 cc.
2. Serum, goat	1000.0 cc.

Preparation:

(1) Prepare nutrient gelatin.

(2) Melt (1) and cool to about 60°C.

(3) Mix equal parts of (2) and goat serum that has been obtained under aseptic conditions and heated to 60°C.

(4) Pour into sterile plates or sterile tubes.

Sterilization: Sterilization of gelatin not specified. See step (3) for sterilization of serum.

Use: Cultivation of fowl diphtheria bacilli. Müller reported that the gelatin was liquified.

Reference: Müller (1906 p. 520).

2322. Beijerinck's Trypsinized Gelatin

Constituents:

1. Water	900.0 cc.
2. Gelatin (liquefied)	10.0 cc.
3. $(NH_4)NO_3$	5.0 g.
4. Potassium phosphate	5.0 g.
5. Gelatin	80.0 g.

Preparation:

(1) Liquefy gelatin with pancreas extract (method not given).

(2) Dissolve 10.0 cc. (1), 3, 4 and 5 in 900.0 cc. of water.

(3) Neutralize, or adjust to a slight acidity.

Sterilization: Not specified.

Use: To study liquefaction of gelatin and pigment production by Chlorococcum. Beijerinck reported that chlorococcum did not liquify the gelatin.

Reference: Beijerinck (1890 p. 461).

2323. Jensen's Pepsinized Milk Gelatin

Same as medium 1112 but solidified by the addition of gelatin.

SUBGROUP III-B. SECTION 3

Basal or complete media containing gelatin and other constituents of unknown chemical composition, but not digests.

A_1. Containing unknown constituents of plant origin only.
B_1. Yeasts or their derivatives employed.
Bobilioff-Preisser's Yeast Water Gelatin.......................... 2324
Beijerinck's Glucose Yeast Gelatin. 2325
Will's Sucrose Yeast Gelatin....... 2326
Beijerinck's Urea Yeast Gelatin.... 2327
B_2. Flowering plants or their derivatives employed.
C_1. Non leguminous plants or derivatives used.
D_1. Grains or their derivatives used.
Will's Basal Wort Gelatin.......... 2328
Heinemann's Beer Wort Gelatin.... 2329
Janke's Alcohol Beer Gelatin....... 2330
D_2. Fruits or their derivatives used.
Perold's Grape Juice Gelatin....... 2331
Dombrowski's Raisin Must Gelatin. 2332
Besson's Raisin Infusion Gelatin... 2333
Klimmer's Prune Infusion Gelatin.. 2334
Löhnis' Plum Extract Gelatin...... 2335
D_3. Tubers or their derivatives used.
Elsner's Iodide Potato Gelatin..... 2336
Holz's Potato Gelatin.............. 2337
Goadby's Glycerol Potato Gelatin (Tanner)......................... 2338
Zikes' Carrot Infusion Gelatin...... 2339
D_4. Non leguminous plants or their derivatives other than D_1 to D_3 used.
Stutzer, Burri and Maul's Glucose Mustard Seed Gelatin............ 2340
Groenwege's Tomato Infusion Gelatin.......................... 2341
Will's Cabbage Juice Gelatin....... 2342
Müller-Thurgaw's Grape Vine Infusion Gelatin................... 2343
C_2. Leguminous plants or derivatives used.
De Rossi's Glucose Leguminous Plant Gelatin................... 2344
Simon's Asparagin Legume Extract Gelatin (Klimmer and Krüger).... 2345
Zipfel's Glucose Legume Infusion Gelatin.......................... 2346
Kaufman's Jequirity Seed Gelatin.. 2347
Stutzer, Burri and Maul's Glucose Alfalfa Gelatin.................. 2348
Barthel's Basal Lupini Extract Gelatin.......................... 2349
A_2. Containing unknown constituent of animal origin only.
B_1. Extracts or infusions not employed.
C_1. Tissues or body fluids used.
Nastinkoff's Egg Yolk Gelatin...... 2350
Deycke and Voigtländer's Albuminate Gelatin.................... 2351
C_2. Secretions or excretions used.
D_1. Milk or its derivatives used.
Raskin's Whey Albumin Gelatin.... 2352
Giltner and Ludum's Amniotic Fluid Gelatin.......................... 2353
Raskin's Whey Gelatin............. 2354
Reinsch's Fat-free Milk Gelatin.... 2355
Abbott's Milk Gelatin.............. 2356
Heinemann's Whey Gelatin......... 2357
Raskin's Casein Whey Gelatin (Peifer)......................... 2358
D_2. Milk or its derivatives not used.
Piorkowski's Urine Infusion Gelatin (Scholz and Krause)............. 2359
B_2. Extracts or infusions employed.
C_1. Infusions specified.
Kutscher's Meat Infusion Gelatin.. 2360
Graham-Smith's Heart Infusion Gelatin.......................... 2361
Beijerinck's Asparagin Meat Infusion Gelatin (Ludwig).......... 2362
Kopp's Tyroid Infusion Gelatin.... 2363
Wroblewski's Suprarenal Capsule Infusion Gelatin................. 2364
Harde and Hauser's Fish Infusion Gelatin.......................... 2365
C_2. Extracts specified.
Molisch's Meat Extract Gelatin.... 2366
Hollborn's Meat Extract Gelatin... 2367
A_3. Containing unknown constituents other than of plant or animal origin.
Fremlin's Soil Infusion Gelatin..... 2368
Conn's Glucose Soil Infusion Gelatin.......................... 2369
Löhnis Cyanamide Soil Infusion Gelatin.......................... 2370
Conn's Tartrate Soil Infusion Gelatin.......................... 2371

2324. Bobilioff-Preisser's Yeast Water Gelatin

Constituents:

1. Yeast water................	1000.0 cc.
2. Gelatin (10.0%)...........	100.0 g.

Preparation:

(1) Dissolve 10.0% gelatin in yeast water.

Sterilization: Not specified.

Use: Cultivation of Oospora, fungi imperfecti. The authors reported that gelatin was liquefied.
Reference: Bobilioff-Preisser (1916 p. 400).

2325. Beijerinck's Glucose Yeast Gelatin

Same as medium 2081 but solidified by the addition of 20.0% gelatin instead of agar.

2326. Will's Sucrose Yeast Gelatin

Constituents:

1. Yeast water	1000.0 cc.
2. Sucrose (6.0%)	60.0 g.
3. Gelatin (10.0%)	100.0 g.

Preparation:
(1) Add 6.0% sucrose and 10.0% gelatin to neutral yeast water.
Sterilization: Not specified.
Use: Cultivation of non-spore forming saccharomyces.
Reference: Will (1908 p. 387).

2327. Beijerinck's Urea Yeast Gelatin

Constituents:

1. Water	1000.0 cc.
2. Yeast (pressed)	200.0 g.
3. Urea	20.0 to 30.0 g.
4. Gelatin	

Preparation:
(1) Prepare yeast water by boiling 200.0 g. pressed yeast with 1000.0 cc. water.
(2) Add 2 or 3.0% urea and gelatin (amount not specified) to (1).
Sterilization: Not specified.
Use: To show iridescence by urea splitting organisms, *Urococcus ureae* and *Urobacillus pastenrii*. Author reported that when a urea splitting organism or a particle of $(NH_4)_2CO_3$ was added to a properly prepared plate, a characteristic precipitate was formed. This was amorphous and stayed at first entirely on the upper surface of the gelatin in a thin sheet. This showed Newton's color rings. As the layer grew it became thicker and the edge spread over a greater surface giving a color ring. After a time a white precipitate formed in the depths of the gelatin, which assumed the shape of plano-convex lens. Non-urea splitting organisms did not show this. Tausz and Peters cultivated paraffin bacteria and Vierling studied urease production by mycobacteria on similar media.
Variants:
(a) Tausz and Peters cultivated *Bacterium aliphaticum, Bacterium aliphaticum liquefaciens*, on the following medium:
 (1) Boil 100.0 g. pressed yeast in 500.0 cc. water.
 (2) Dissolve 50.0 g. of gelatin in (1).
 (3) Adjustment of reaction not given.
 (4) Sterilize on 3 successive days in streaming steam.
 (5) To determine the production of urease, add 1.25 g. sterile urea to the medium.
(b) Vierling studied urease production by mycobacteria on a medium prepared as follows:
 (1) Boil 200.0 g. pressed yeast with 1000.0 cc. of water.
 (2) Cool, decant and filter.
 (3) Dissolve 25.0 g. urea and 100.0 g. gelatin in the filtrate.
 (4) Sterilize (Method not given).
 (5) Pour in plates.
References: Beijerinck (1901 p. 39), Tausz and Peters (1919 p. 49), Vierling (1920 p. 205).

2328. Will's Basal Wort Gelatin

Constituents:
1. Wort Gelatin (10.0%).
Preparation:
(1) Prepare 10.0% wort gelatin.
(2) Dissolve one of the added nutrients in (1).
Sterilization: Not specified.
Use: Cultivation of mycoderma-like organisms. Park, Williams and Krumwiede used a similar medium for the cultivation of molds.
Added nutrients: The author added 0.7% asparagin or 1.0% ammonium tartrate.
Variants:
(a) Will used the 10.0% wort gelatin without any additions.
(b) Park, Williams and Krumwiede added 2.0% of any desired carbohydrate to beerwort, solidified by the addition of 10.0% gelatin.
References: Will (1900 p. 597), (1910 p. 3), Park, Williams and Krumwiede (1924 p. 134).

2329. Heinemann's Beer Wort Gelatin

Constituents:

1. Beerwort	1000.0 cc.
2. Gelatin (10.0%)	100.0 g.

Preparation:

(1) Autoclave beerwort at 120° for 5 minutes.
(2) Cool.
(3) Filter.
(4) Dissolve 10.0% gelatin in the filtrate.
(5) Clarify with the white of egg.

Sterilization: Not specified.

Use: General culture medium.

Variants:

(a) Nakazawa cultivated yeast on a medium prepared by solidifying 12.0% unhopped beer wort with 10.0% gelatin.
(b) Janke cultivated acetic acid bacteria from beers in a medium prepared as follows:
  (1) Dissolve 10.0 or 12.0% gelatin in hopped lager beer wort.
  (2) Clarify with egg albumin.
  (3) Filter thru paper.
  (4) Distribute in 50.0 cc. lots in flasks.
  (5) Sterilize the discontinuous method in the steamer.
(c) Tanner gave the following method of preparation:
  (1) Measure out 900.0 cc. of beer wort in a sterile flask.
  (2) Add 100.0 g. (10.0%) gelatin to (1).
  (3) Bubble live steam thru to dissolve the gelatin.
  (4) Cool to 60°C.
  (5) Clarify with egg.
  (6) Filter.
  (7) Tube.
  (8) Sterilization not specified.
(d) Klimmer cultivated yeast and molds on the following medium:
  (1) Obtain beer wort from a brewery.
  (2) Add gelatin (amount not given).
  (3) Boil and filter.
  (4) Distribute as desired.
  (5) Sterilize.

References: Heinemann (1905 p. 128), Nakazawa (1909 p. 530), Dombrowski (1910 p. 380), Janke (1916 p. 6), Tanner (1919 p. 56), Klimmer (1923 p. 207), Park, Williams and Krumwiede (1924 p. 134).

2330. Janke's Alcohol Beer Gelatin

Constituents:

1. Lager beer	1000.0 cc.
2. Gelatin (10.0 to 12.0%)	100.0 to 120.0 g.
3. Alcohol (3.0%)	30.0 cc.

Preparation:

(1) Evaporate lager beer to one-half its original volume by steaming.
(2) Add water to its original volume.
(3) Dissolve 10.0% to 12.0% gelatin in (2).
(4) Clarify with white of egg.
(5) Filter thru folded filter.
(6) Make up to the original volume by the addition of water.
(7) Distribute in 10.0 cc. lots in Freudenreich flasks.
(8) Add 3.0% by volume of absolute alcohol.

Sterilization: Sterilize on 3 successive days in the steamer.

Use: Cultivation of acetic acid bacteria from beers.

Reference: Janke (1916 p. 6).

2331. Perold's Grape Juice Gelatin

Constituents:

1. Water	1000.0 cc.
2. Gelatin	150.0 g.
3. Grape juice	60.0 cc.

Preparation:

(1) Dissolve 150.0 g. gelatin in 1 liter of water.
(2) Clarify the gelatin with egg white.
(3) To each 100.0 cc. of (2) add 6.0 cc. of grape juice.

Sterilization: Sterilize for one hour in the steamer.

Use: Cultivation of bacteria from wine.

Reference: Perold (1909 p. 18).

2332. Dombrowski's Raisin Must Gelatin

Same as medium 2109 but solidified by the addition of 10.0% gelatin instead of 1.5% agar.

2333. Besson's Raisin Infusion Gelatin

Constituents:

1. Water	1000.0 cc.
2. Raisins	250.0 g.
3. Gelatin	100.0 g.
4. Sodium phosphate	

Preparation:

(1) Soak 250.0 g. of dried raisins in a liter of water for several hours.
(2) Boil.
(3) Pass thru a coarse sieve.
(4) Filter thru paper.
(5) Dissolve 100.0 g. gelatin and a pinch of sodium phosphate in the filtrate.
(6) Boil for 2 or 3 minutes.
(7) Make slightly alkaline by the addition of soda.
(8) Heat at 115° for 5 minutes.
(9) Filter thru paper, in the autoclave or use a hot water funnel.
(10) Tube.

Sterilization: Sterilize at 110° for 20 minutes.

Use: General culture medium.

Reference: Besson (1920 p. 40).

2334. Klimmer's Prune Infusion Gelatin

Constituents:

1. Water	500.0 cc.
2. Prunes	100.0 g.
3. Gelatin	

Preparation:

(1) Boil 100.0 g. of dried prunes in 500.0 cc. of water.
(2) Filter.
(3) Add gelatin (amount not given).
(4) Boil.
(5) Filter.
(6) Distribute as desired.

Sterilization: Method not given.

Use: General culture medium.

Reference: Klimmer (1923 p. 206).

2335. Löhnis' Plum Extract Gelatin

Constituents:

1. Water	1000.0 cc.
2. Plums	1000.0 g.
3. Gelatin (10.0%)	100.0 g.

Preparation:

(1) Boil 1000.0 g. of plums with 1000.0 cc. of water twice.
(2) Add 10.0% gelatin to the juice of (1) without neutralization.

Sterilization: Not specified.

Use: Cultivation of molds.

Reference: Löhnis (1918 p. 88).

2336. Elsner's Iodide Potato Gelatin

Constituents:

1. Water	1000.0 cc.
2. Potato	500.0 g.
3. Gelatin	
4. KI (1.0%)	10.0 g.

Preparation:

(1) Prepare a gelatin using 500.0 g. potatoes and 1 liter of water. Amount of gelatin to be added or method of preparation not given.
(2) Add N/1 NaOH to neutralize to litmus.
(3) Filter and flask.
(4) Add 1.0% KI to each flask of sterile (3).

Sterilization: Method of sterilization of (3) not given.

Use: Differentiation of coli and typhoid bacteria. The author reported that *B. coli* grew rapidly and luxuriantly. Colonies were brown. Typhoid organisms grew slower. After 48 hours colonies were small and glistening resembling drops of water.

Variants:

(a) Grimbert gave the following method of preparation:
  (1) Grate 500.0 g. potatoes and allow to macerate with one liter of water for 3 or 4 hours or better allow to stand over night in the cold.
  (2) Decant and filter.
  (3) Autoclave the filtrate for 10 minutes.
  (4) Filter.
  (5) Add 15.0 g. of gelatin for each 100.0 cc. of the filtrate, and melt by heating on the salt water bath.
  (6) Cool to 55°C. and add the white of an egg suspended in a little water.
  (7) Adjust the reaction so that 5.0 cc. of lime solution is required to neutralize 10.0 cc. medium to phenolphthalein. (An acidity equivalent to 1.0 g. H_2SO_4 per liter.)
  (8) Heat in the autoclave for 10 minutes at 110°C. to clarify.
  (9) Filter.
  (10) Tube in 9.0 cc. lots.
  (11) Sterilize in the autoclave at 110° for 15 minutes.
  (12) When ready for use add 1.0 cc. of a sterile 10.0% KI solution to each tube.

(b) Migula prepared the medium as follows:
   (1) Prepare potato infusion from 1 liter of water and 500.0 g. of potato.
   (2) Boil (1) with nutrient gelatin (amount not specified).
   (3) Add 2.5 to 3.0 cc. of 0.1 normal NaOH to each 10.0 cc. of (2).
   (4) Filter.
   (5) Sterilize (method not given).
   (6) When ready for use, pour into a flask, add 1.0% KI and the water under investigation.
   (7) Pour into plates.

(c) Smith prepared the medium as follows:
   (1) Grate 500.0 g. of pared potatoes and place the pulp in the refrigerator in a porcelain dish over night.
   (2) Express the juice from the pulp.
   (3) Filter several times thru a layer of absorbent cotton or thru animal charcoal.
   (4) The filtrate should now be titered with decinormal sodium hydroxide solution to determine its reaction and the amount of water which will be required to be added to reduce its acidity to the standard. In this quantity of water is now boiled the amount of gelatin required to make 10.0% proportion of the gelatin when the potato juice shall have been added, the reaction of the gelatin being corrected to neutral point after it has been dissolved (and before adding the potato juice) by means of normal sodium hydroxide solution. This done and the bulk of the fluid corrected for evaporation to that necessary to properly dilute the potato juice the latter is slowly added to the gelatin and mixed, and the whole boiled for 5 to 10 minutes.
   (5) Filter.
   (6) Tube.
   (7) Sterilize by the intermittent method.

(d) Ball gave the preparation of the medium as follows:
   (1) Mash 500.0 g. of peeled and washed potatotes, and press thru a fine cloth.
   (2) Allow the juice to settle.
   (3) Filter.
   (4) Cook for one hour.
   (5) Add 10.0% gelatin to (4).
   (6) Add 2.5 cc. of 1/10 normal NaOH and 1.0% KI.
   (7) Sterilization not specified.

(e) Bezançon gave the following method of preparation:
   (1) Peel and grate 500.0 g. of potatoes.
   (2) Add (1) to a liter of water and allow to soak for several hours.
   (3) Allow to settle.
   (4) Decant and filter.
   (5) Autoclave for 10 minutes.
   (6) Add 150.0 g of gelatin and melt on a salt water bath.
   (7) Adjust the reaction to a slight acidity.
   (8) Sterilize at 150°C.
   (9) Filter.
   (10) Flask in 100.0 cc. lots.
   (11) When ready for use add 1.0 g. of KI and distribute in tubes.
   (12) Final sterilization not given.

References: Elsner (1896 p. 29), Grimbert (1896 p. 723), Migula (1901 p. 23), Smith (1902 p. 78), Ball (1919 p. 82), Bezançon (1920 p. 340).

2337. Holz's Potato Gelatin

Constituents:
1. Potatoes.
2. Gelatin.

Preparation:
   (1) Thoroly wash potatoes.
   (2) Cut out the eyes and all rotten parts of the potatoes.
   (3) Peel and wash the potatoes in tap water.
   (4) Rub the potatoes to a pulp on an ordinary kitchen grater.
   (5) Filter the juice thru a clean towel and press the pulp free from juice.
   (6) Place the juice in a flask, plug with cotton and allow to stand for 24 hours at 10°C.
   (7) Filter, the juice has turned brown.
   (8) Heat in the steamer for 30 minutes and filter again.

(9) Add 50.0 g. gelatin to 400.0 cc. of the filtrate, and heat for 45 minutes in the steamer.
(10) Tube in 5.0 or 10.0 cc. lots in sterile test tubes.
(11) The medium is clear and brown. The reaction is acid. 10.0 g. requires 1.6 cc. N/1 alkali to neutralize to litmus.

Sterilization: Sterilize on 3 successive days for 15 minutes each day in streaming steam.

Use: Isolation of *Bacillus typhosus* and *Bacillus coli communis.*

Variants:

(a) Holz added 0.05% phenol to inhibit molds, liquefiers and other forms.
(b) Smith prepared the medium as follows:
  (1) Grate 500.0 g. of pared potato and place the pulp in the refrigerator in a porcelain dish over night.
  (2) Express the juice from the pulp.
  (3) Filter several times thru a layer of absorbent cotton or thru animal charcoal.
  (4) The filtrate should now be titered with decinormal sodium hydroxide solution to determine its reaction and the amount of water which will be required to be added to reduce its acidity to the standard. In this quantity of water is now boiled the amount of gelatin required to make 10.0% proportion of the gelatin when the potato juice shall have been added, the reaction of the gelatin being corrected to neutral point after it has been dissolved (and before adding the potato juice) by means of normal sodium hydroxide solution. This done and the bulk of the fluid corrected for evaporation to that necessary to properly dilute potato juice, the latter is slowly added to the gelatin and mixed, and the whole boiled for 5 to 10 minutes.
  (5) Filter.
  (6) Tube.
  (7) Sterilize by the intermittent method.
(c) Zikes (Will) prepared a medium as follows:
  (1) Peel clean potatoes and grind in the grinding machine.
  (2) Press out the juice by means of a hand press.
  (3) Add bone black and heat and finally filter while hot.
  (4) Add 10.0% gelatin and prepare the potato water gelatin in the usual manner. (Method not given.) (Carrots may be handled in the same way. Heat the carrot juice in the steamer before filtering.)
(d) Elsner (Tanner) gave the following method:
  (1) Peel and grate 500.0 g. of potato.
  (2) Grind (1) and soak in distilled water for 12 hours.
  (3) Strain and allow to stand.
  (4) Filter to clear.
  (5) Make up to one liter.
  (6) Dissolve 15.0% gelatin in (5).
  (7) Adjust the reaction to acid (indicator not specified).
  (8) Sterilize (method not given).
(e) Elsner (Besson) prepared the medium as follows:
  (1) Thoroly wash, grate and peel potatoes.
  (2) Soak 500.0 g. of (1) in a liter of water for 4 hours.
  (3) Pass thru a sieve.
  (4) Allow to settle over night.
  (5) Decant.
  (6) Make up the decanted liquid to 1000.0 cc.
  (7) Dissolve 150.0 to 200.0 g. of gelatin in (6) by heating gently.
  (8) Boil for several minutes.
  (9) Add soda solution until the reaction is but only slightly acid.
  (10) Heat at 115° for 5 minutes.
  (11) Filter thru paper, in the autoclave or use a hot water funnel.
  (12) Tube.
  (13) Sterilize at 110° for 20 minutes.

References: Holz (1890 p. 159), Smith (1902 p. 78), Will (1907 p. 435), Tanner (1919 p. 60), Besson (1920 p. 40).

2338. Goadby's Glycerol Potato Gelatin (Tanner)

Constituents:

1. Glycerol potato broth	1000.0 cc.
2. Gelatin	100.0 g.

Preparation:
- (1) Prepare glycerol potato broth.
- (2) Add 100.0 g. of gelatin to (1).
- (3) Boil.
- (4) Adjust the reaction.
- (5) Filter.

Sterilization: Method not given.

Use: General culture medium.

Reference: Tanner (1919 p. 60).

2339. Zikes' Carrot Infusion Gelatin

Same as variant (c) medium 2337 but using carrots instead of potatotes.

2340. Stutzer, Burri and Maul's Glucose Mustard Seed Gelatin

Constituents:

1. Water	1000.0 cc.
2. White mustard seed	400.0 g.
3. Glucose	20.0 g.
4. Gelatin	100.0 g.

Preparation:
- (1) Germinate white mustard seed at 30°C. for 3 days. The sprouts are from 1 to 1.5 cm. long.
- (2) Add 1 liter of water to 400.0 g. of (1).
- (3) Steam at 100°C. for 2 hours.
- (4) Filter while hot.
- (5) Mix 500.0 cc. of filtrate with an equal volume of water.
- (6) Dissolve 100.0 g. gelatin and 20.0 g. of glucose in (5).
- (7) Finish the preparation in the usual manner. (Method not specified.)
- (8) Adjust the reaction to a slight acidity.
- (9) Distribute in 8.0 cc. lots.

Sterilization: Not specified.

Use: Cultivation of *Bacillus radicicola* and other nodule bacteria.

Reference: Stutzer, Burri and Maul (1896 p. 667).

2341. Groenewege's Tomato Infusion Gelatin

Constituents:

1. Water	100.0 cc.
2. Tomato	20.0 g.
3. Gelatin (10.0%)	10.0 g.

Preparation:
- (1) Boil 20.0 g. of the fresh material of ripe tomatoes in 100.0 cc. of water.
- (2) Filter.
- (3) Neutralize the filtrate to litmus by the addition of Na_2CO_3.
- (4) Add 10.0% gelatin.
- (5) Clarify and filter.

Sterilization: Not specified.

Use: Cultivation of *Phytibacter lycopersicum* (causing tomato rot).

Reference: Groenewege (1917 p. 24).

2342. Will's Cabbage Juice Gelatin

Constituents:
1. Cabbage juice.
2. Gelatin.

Preparation:
- (1) Press the juice from cabbage leaves.
- (2) Solidify (1) by the addition of 10.0% gelatin.

Sterilization: Not specified.

Use: Cultivation of mycoderma.

Reference: Will (1910 p. 3).

2343. Müller-Thurgau's Grape Vine Infusion Gelatin

Same as medium 1188 but solidified by the addition of gelatin.

2344. de Rossi's Glucose Leguminous Plant Gelatin

Constituents:

1. Water	1000.0 cc.
2. Leguminous plant	
3. Gelatin	
4. Glucose (2.0%)	

Preparation:
- (1) Prepare a gelatin from the extract of leguminous plant leaves. (Method not given.)
- (2) Add 2.0% glucose.
- (3) Distribute in tubes.

Sterilization: Method not given.

Use: To study nitrogen fixation.

Reference: de Rossi (1909, 1910 p. 271).

2345. Simon's Asparagin Legume Extract Gelatin (Klimmer and Krüger)

Constituents:

1. Water	1000.0 cc.
2. Gelatin	110.0 g.
3. Legume extract	2.0 cc.
4. Asparagin	2.0 g.
5. Glucose	10.0 g.
6. Malic acid	

Preparation:
- (1) Prepare the legume extract by pouring hot water over finely chopped straw of the legumes, mix well,

macerate for 2 days and then boil an hour. Pour off the liquid, filter and evaporate until it contains 10.0% dry material.
(2) Dissolve 2, 3, 4 and 5 in 1.
(3) Add 2.0 cc. of (1) to (2).
(4) Neutralize by the addition of NaOH using litmus as an indicator.
(5) Add malic acid to give a distinct acid reaction.

Sterilization: Not specified.

Use: Cultivation of nodule bacteria from leguminous plants.

Variants: Klimmer prepared the medium using citric acid to adjust the reaction instead of malic acid.

References: Klimmer and Krüger (1914 p. 258), Klimmer (1923 p. 228).

2346. Zipfel's Glucose Legume Infusion Gelatin

Same as medium 2140 but solidified with 150.0 g. gelatin instead of 30.0 g. of agar.

2347. Kaufman's Jequirity Seed Gelatin

Same as medium 2132 but solidified with 15.0% gelatin instead of 1.5 to 2.0% agar.

2348. Stutzer, Burri and Maul's Glucose Alfalfa Gelatin

Constituents:

1. Water	1000.0 cc.
2. Alfalfa	400.0 g.
3. Glucose	20.0 g.
4. Gelatin	100.0 g.
5. Na_2CO_3	1.0 g.

Preparation:
(1) Pour 1 liter of water over 400.0 g. green alfalfa without roots.
(2) Add 20.0 g. glucose.
(3) Steam for 90 minutes.
(4) Filter.
(5) Add 100.0 g. gelatin to the filtrate and proceed to prepare a sterile medium in the usual manner. (Method not specified.)
(6) Do not neutralize but add 1.0 g. Na_2CO_3 (Reaction slightly acid).
(7) Tube in 8.0 cc. lots.

Sterilization: Not specified

Use: Cultivation of *Bacillus radicicola* and other nodule bacteria.

Reference: Stutzer, Burri and Maul (1896 p. 666).

2349. Barthel's Basal Lupini Extract Gelatin

Constituents:

1. Water	900.0 cc.
2. Gelatin	100.0 g.
3. Sucrose	5.0 g.
4. Lupine extract	2.5 cc.

Preparation:
(1) Dissolve 2, 3 and 4 in 1.
(2) Add one of the added nutrients.
(3) Tube in 10.0 cc. lots.

Sterilization: Sterilize the fractional method.

Use: To study formation of bacteroids by *Bacillus radicicola* and other legumes.

Added nutrients: The author used the following materials:
(a) 0.1, 0.3 or 0.5%, caffeine ground up with water and gum arabic.
(b) 0.1, 0.3 or 0.5 g. guanine ground up with a little water and gum arabic.
(c) 0.1, 0.3 or 0.5 g. guanidine from a watery solution. Acidify slightly by the addition of tartaric acid.
(d) 0.1, 0.3 or 0.5% pyridine from a watery solution.
(e) 0.1, 0.3 or 0.5% chinoline from an alcoholic solution.

Reference: Barthel (1921 p. 638).

2350. Nastiukoff's Egg Yolk Gelatin

Same as medium 2144 but solidified with 80.0 to 100.0 g. gelatin instead of 15.0 to 20.0 g. agar.

2351. Deycke and Voigtländer's Albuminate Gelatin

Same as medium 2034 but solidified with gelatin instead of agar.

2352. Raskin's Whey Albumin Gelatin

Constituents:

1. Whey	1000.0 cc.
2. Albumin	100.0 g.
3. Gelatin	60.0 to 100.0 g.

Preparation:
(1) Stir thoroly with a glass rod, the albumin of fresh eggs in a flat bottomed dish, and add, drop by drop, concentrated NaOH until a solid opaque gelatinous material is obtained.

(2) Cut this solid egg albumin in small pieces and place in distilled water. Do not allow the alkaline albumin to stand or it will liquefy in several hours.
(3) Shake thoroly and pour off the water. Continue this washing until the wash water is only slightly alkaline.
(4) Place the coagulated albuminate in a flask of distilled water, plug with cotton and heat in the steamer for 15 to 30 minutes. This dissolves the albumin.
(5) Filter. This is to be a saturated solution of albumin.
(6) Add 10.0% (6) to medium 2274 instead of 1.0% peptone.
(7) Neutralize with soda.
(8) Filter until clear thru paper in a hot water funnel.

Sterilization: Not specified.

Use: Cultivation of pathogenic bacteria.

Variants:

(a) The author added 0.5% NaCl.
(b) Peifer gave the following method of preparation:
  (1) Heat fresh milk to 60 to 70°C.
  (2) Dissolve 60.0 to 70.0 g. gelatin in 1000.0 cc. of (1).
  (3) Boil until the casein is completely coagulated.
  (4) Strain thru a linen towel.
  (5) Pour in a suitable container so that the fat may raise.
  (6) Remove the fat that comes to the top.
  (7) Add 1.0% of a sodium albuminate powder solution and soda to neutralize.
  (8) Method of sterilization not given.

References: Raskin (1887 p. 359), Peifer (1888 p. 568).

2353. Giltner and Ludlum's Amniotic Fluid Gelatin

Constituents:

1. Amniotic fluid.
2. Gelatin.

Preparation:

(1) Solidify amniotic fluid with gelatin.

Sterilization: Method not given.

Use: General culture medium.

Reference: Giltner and Ludlum (1916 pp. 91–92).

2354. Raskin's Whey Gelatin

Same as medium 2161 but solidified with 12.0% gelatin instead of 1.75% agar.

2355. Reinsch's Fat-free Milk Gelatin

Constituents:

1. Water	100.0 cc.
2. Milk	500.0 cc.
3. Gelatin	20.0 g.

Preparation:

(1) Dissolve 20.0 g. of gelatin in 100.0 cc. water.
(2) Place 500.0 cc. of fresh cow milk in a separatory funnel and add 1.0 g. NaOH (2.5 cc. solution of 400.0 g. NaOH in a liter).
(3) Shake well and allow to stand at about 18°C. for 48 hours.
(4) Remove the nearly transparent milk from the bottom of the funnel and add it to a second separatory funnel.
(5) Add 250.0 cc. ether and shake well.
(6) Allow to stand for 48 hours.
(7) Place the opalescent liquid now in a large sterile flask, plug with cotton and heat to 50°C.
(8) Place under the receiver of a water suction pump for 3 or 4 hours until all the ether is evaporated.
(9) Add about 0.2% NaOH in order that the acid gelatin does not cause a precipitate of casein.
(10) Add two to three parts of (9) to one part (1).
(11) Distribute in sterile test tubes.

Sterilization: Not specified.

Use: General culture medium. The author reported that the medium was too alkaline to support the growth of most bacteria.

Reference: Reinsch (1892 p. 32).

2356. Abbott's Milk Gelatin

Constituents:

1. Milk	1000.0 cc.
2. Gelatin (10.0 to 12.0%)	100.0 to 120.0 g.

Preparation: (1) Solidify milk by the addition of 10.0 to 12.0% gelatin.

Sterilization: Not specified.

Use: General culture medium.

Reference: Abbott (1921 p. 139).

2357. Heinemann's Whey Gelatin

Constituents:

1. Whey	1000.0 cc.
2. Gelatin (10.0%)	100.0 g.

Preparation: (1) Solidify clarified whey by the addition of 10.0% gelatin.

Sterilization: Not specified.

Use: General culture medium.

Variants:

- (a) Bobilioff-Preisser cultivated Oospora on a similar medium. They reported that the gelatin was liquefied.
- (b) Meier made bacterial counts of milk and whey on a medium prepared as follows:
  - (1) Dissolve 110.0 g. gelatin and 5.0 g. NaCl in 500.0 cc. water.
  - (2) Mix equal parts of whey and (1).
  - (3) Neutralize by the addition of KOH. Add KOH until turmeric paper is turned quite weakly brownish red.
  - (4) Sterilization not specified
- (c) Klimmer prepared the medium as follows:
  - (1) Coagulate the casein from milk by heating at 40°C. in the presence of rennet.
  - (2) Separate the whey from the casein.
  - (3) Solidify by the addition of gelatin.
  - (4) Sterilization not specified.

References: Heinemann (1905 p. 128), Bobilioff-Preisser (1916 p. 400), Meier (1918 p. 435), Tanner (1919 p. 55), Klimmer (1923 p. 172).

2358. Raskin's Casein Whey Gelatin
(Peifer)

Constituents:

1. Water	150.0 cc.
2. Casein (8.0%)	12.0 g.
3. Whey	350.0 cc.
4. Gelatin	42.0 g.

Preparation:

- (1) Prepare an 8.0% solution of pure fat-free casein.
- (2) Prepare a filtered mixture of whey with 12.0% gelatin.
- (3) Mix 150.0 cc. of (1) with 350.0 cc. of (2).
- (4) Heat to 60°C. for 15 minutes.
- (5) Distribute into sterile containers.

Sterilization: Not specified.

Use: Cultivation of pathogenic organisms.

References: Raskin (1887 #43), Abst. by Peifer (1888 p. 568).

2359. Piorkowski's Urine Infusion Gelatin
(Scholz and Krause)

Constituents:

1. Urine.
2. Meat water gelatin.

Preparation:

- (1) Collect urine under aseptic conditions from a healthy individual. Specific weight of the urine to be 1.015 to 1.020. It is slightly alkaline or neutral in reaction.
- (2) Inoculate with a loop of *Micrococcus* urea (liquefac. or non liquefac.) from a glycerol agar culture.
- (3) Incubate for 24 to 48 hours at 22°C.
- (4) After this time the reaction is slightly alkaline.
- (5) Filter.
- (6) Distribute in tubes in 5 to 10.0 cc. lots.
- (7) Add to each tube of sterile (6) 5.0 to 10.0 cc. of a sterile 10.0% meat water gelatin (exact method of preparation not given) under aseptic conditions.

Sterilization: Sterilize (6) in the steamer at 100°C. on 3 successive days for 10 minutes. Method of sterilization of gelatin not given.

Use: Diagnosis of typhoid fever.

Reference: Scholz and Krause (1900 p. 432).

2360. Kutscher's Meat Infusion Gelatin

Constituents:

1. Meat Infusion	1000.0 cc.
2. Gelatin (10.0%)	100.0 g.

Preparation:

- (1) Prepare meat infusion.
- (2) Dissolve 10.0% gelatin in 1 by boiling over a free flame.
- (3) Add soda solution until the reaction is only slightly alkaline.
- (4) Filter.

Sterilization: Method of sterilization not given.

Use: Isolation and cultivation of *Spirillum undula majris*. Also used by other investigators in making bacterial counts.

Variants:

- (a) Fuller and Johnson made bacterial counts in water analysis on a medium prepared by dissolving 120.0 g. of gelatin in 1000.0 cc. of meat infusion (contains no peptone or salts).

(b) Meier used the following preparation for the bacterial count of milk:

(1) Boil 500.0 g. of fat and tendon free beef in 1 liter water.
(2) Filter.
(3) Dissolve 10.0 g. gelatin and 5.0 g. NaCl in the filtrate.
(4) Neutralize by the addition of KOH. Add KOH until turmeric paper is turned quite weakly brownish red.
(5) Sterilization not specified.

References: Kutscher (1895 p. 615), Fuller and Johnson (1899–1900 p. 85), Meier (1918 p. 435).

2361. Graham-Smith's Heart Infusion Gelatin

Same as medium 1342 but solidified with gelatin.

2362. Beijerinck's Asparagin Meat Infusion Gelatin (Ludwig)

Constituents:

1. Sea water meat infusion....	1000.0 cc.
2. Glycerol....................	10.0 g.
3. Asparagin..................	5.0 g.
4. Gelatin....................	80.0 g.

Preparation:

(1) Exact method of preparation of meat infusion not given. To be prepared from sea water.
(2) Dissolve 2, 3 and 4 in (1).

Sterilization: Method not given.

Use: Cultivation of photogenic bacteria. (*Ph. Pfugeri, Ph. phosphorescence, Ph. indicum and Ph. luminosum*).

References: Ludwig (1890 p. 617), Beijerinck (1890 p. 617).

2363. Kopp's Thyroid Infusion Gelatin

Constituents:

1. Water......................	1000.0 cc.
2. Thyroid glands, sheep......	500.0 g.
3. Gelatin....................	100.0 g.
4. NaCl......................	5.0 g.

Preparation:

(1) Free sheep thyroid glands from fat and chop the glands into small pieces.
(2) Extract the glands for 3 hours with an equal weight of sterile water.
(3) Add (2) to moistened linen and press the fluid thru.
(4) Prepare a 20% watery gelatin solution containing 1.0% NaCl.
(5) Mix equal volumes of (4) and sterile (3).

Sterilization: Filter (3) thru a clay filter to sterilize. Sterilization of gelatin not given.

Use: Cultivation of colon typhoid bacteria.

Reference: Kopp (1895 p. 81).

2364. Wroblewski's Suprarenal Capsule Infusion Gelatin

Constituents:

1. Water......................	500.0 cc.
2. Suprarenal capsule (ox).....	100.0 g.
3. Gelatin....................	60.0 g.
4. NaCl......................	3.0 g.

Preparation:

(1) Remove the suprarenal capsule from an ox immediately after death.
(2) Chop into small bits and rub to a pulp.
(3) To 100.0 g. of (2) add 200.0 cc. of water.
(4) Make (3) alkaline with soda and boil for 2 hours. (Filtration not specified.)
(5) Dissolve 60.0 g. of gelatin and 3.0 g. NaCl in 300.0 cc. of water.
(6) Mix equal quantities of (5) and (4).
(7) Sterilize. (Method not given.)
(8) Filter 2 or 3 times to clarify.
(9) Sterilize again (method not specified).

Sterilization: Sterilize (9) method not given. Store at 36°C. for several days to test sterility.

Use: Cultivation of *Bact. coli, Bact. cholerae asiat.*, anthrax, prodigiosus and pyocyaneus.

Reference: Wroblewski (1896 p. 529).

2365. Harde and Hauser's Fish Infusion Gelatin

Same as medium 1235 but solidified with 15.0% gelatin.

2366. Molisch's Meat Extract Gelatin

Constituents:

1. Water......................	1000.0 cc.
2. Meat extract...............	0.5 g.
3. Gelatin (4.5%).............	45.0 g.

Preparation: (1) Dissolve 2 and 3 in 1.

Sterilization: Not specified.

Use: Cultivation of iron bacteria, *Cladothrix dichotoma* Cohn.

Variants: Zikes used 0.5% meat extract.

References: Molisch (1910 p. 31), Linde (1913 p. 373), Zikes (1915 p. 543), Besson (1920 p. 40).

2367. Hollborn's Meat Extract Gelatin

Constituents:

1. Water	1000.0 cc.
2. Meat extract	16.0 g.
3. Gelatin	100.0 g.
4. K_3PO_4	1.0 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Make weakly alkaline by the addition of NaOH.

Sterilization: Method of sterilization not given.

Use: Cultivation of organism causing Alopecia areata (baldness) *Trichophyton todens.*

Reference: Hollborn (1895 p. 110).

2368. Fremlin's Soil Infusion Gelatin

Constituents:

1. Soil infusion	1000.0 cc.
2. Gelatin (10.0%)	100.0 g.

Preparation:

(1) Prepare soil infusion.

(2) Dissolve 10.0% gelatin in (1).

Sterilization: Method not given.

Use: Cultivation of nitroso bacteria.

Variants: Löhnis prepared the medium as follows:

(1) Heat 1000.0 g. of good rich garden soil with a liter of tap water for 30 minutes in the autoclave under pressure of 1 atmosphere or boil with 2 liters of water over a free flame.

(2) Pour off the turbid liquid.

(3) Mix talc with the liquid.

(4) Filter thru a double filter paper.

(5) Make up the volume to 800.0 cc. if necessary.

(6) Solidify by the addition of gelatin.

(7) Sterilize. (Method not given.)

References: Fremlin (1903 p. 373), Löhnis (1913 p. 101).

2369. Conn's Glucose Soil Infusion Gelatin

Constituents:

1. Water	1000.0 cc.
2. Gelatin (12.0%)	120.0 g.
3. Soil infusion (20.0%)	200.0 cc.
4. Glucose (0.1%)	1.0 g.

Preparation:

(1) Prepare soil infusion by heating soil for one hour at one atmosphere pressure, mix with twice its weight of water, allow to stand over night cold, boil 30 minutes and filter. Dilute to contain 0.12 to 0.14% total solids, if necessary.

(2) Dissolve 12.0% gelatin, 20.0% soil extract and 0.1% glucose in water.

(3) Add NaOH to adjust the reaction to +0.5 to phenolphthalein.

Sterilization: Not specified.

Use: Bacterial count of soils.

Variants: Conn prepared the medium as follows:

(1) Heat soil in an autoclave at 20 to 25 pounds pressure.

(2) Mix (1) with an equal volume of distilled water and allow to stand in the cold for 12 hours.

(3) Boil 30 minutes.

(4) Make up the water lost by evaporation.

(5) Filter.

(6) Dilute with distilled water to $\frac{1}{10}$ its natural strength (900.0 cc. distilled water 100.0 cc. extract).

(7) Dissolve 120.0 g. Gold Label Gelatin in 1000.0 cc. of (6).

(8) Clarify by the use of egg white.

(9) Adjust to 0.5% normal acid using phenolphthalein as indicator.

(10) Add 1.0 g. of glucose.

(11) Tube.

(12) Sterilization not given.

References: Conn (1910 p. 425), (1911–12 p. 71), (1914 p. 512), (1916 p. 722), Tanner (1919 p. 55).

2370. Löhnis Cyanamide Soil Infusion Gelatin

Constituents:

1. Water	1000.0 cc.
2. Soil	1000.0 g.
3. Calcium cyanamide (0.2%)	1.6 g.
4. Asparagin (0.01%)	0.08 g.
5. Glucose (0.01%)	0.08 g.
6. K_2HPO_4 (0.05%)	0.4 g.
7. Gelatin (10.0%)	80.0 g.

Preparation:

(1) Heat 1000.0 g. of good rich garden soil with a liter of tap water for 30 minutes in the autoclave under pressure of 1 atmosphere or boil with 2 liters of water over a free flame.
(2) Pour off the turbid liquid.
(3) Mix talc with the liquid.
(4) Filter thru a double filter paper.
(5) Make up the volume to 800.0 cc. if necessary.
(6) Dissolve 3, 4, 5, 6 and 7 in (5).
(7) Tube. The reaction must be distinctly alkaline.

Sterilization: Not specified.

Use: Cultivation of cyanamide decomposers.

Reference: Löhnis (1913 p. 108).

2371. Conn's Tartrate Soil Infusion Gelatin

Constituents:

1. Water	1000.0 cc.
2. Gelatin (12.0%)	120.0 g.
3. Soil infusion (20.0%)	200.0 cc.
4. Glucose (0.1%)	1.0 g.
5. Ammonium tartrate (0.1%)	1.0 g.

Preparation:

(1) Prepare soil infusion by heating soil for one hour at one atmosphere pressure, mix with twice its weight of water, allow to stand over night cold, boil 30 minutes and filter. Dilute, to contain 0.12 to 0.14% total solids, if necessary.
(2) Dissolve 12.0% gelatin, 20.0% soil infusion, 0.1% glucose and 0.1% ammonium tartrate in water.
(3) Add NaOH to adjust the reaction to +0.5 to phenolphthalein.

Sterilization: Not specified.

Use: Bacterial count in soil.

Reference: Conn (1910 p. 425).

GROUP IV. REVERSIBLY SOLID MEDIA OTHER THAN AGAR OR GELATIN

A_1. Additional constituents, if any, of known chemical composition.

Marpman's Chondrin Medium...... 2372
Lehmann's Caragheen Medium..... 2373
Giltner's Butter Medium........... 2374

A_2. Containing additional constituents of unknown chemical composition.

B_1. Only one additional constituent of unknown chemical composition.

Mortensen's Gum Arabic Media.... 2375
Schluter's Basal Isinglass Medium. 2376
Marpmann Peptone Plant Tissue Medium......................... 2377
Matzuschita's Peptone Konbu Medium......................... 2378
Puccinelli's Infusion Fucus Medium 2379
Jacobi's Peptone Fucus Medium.... 2380
Jacobi's Infusion Broth Fucus Medium......................... 2381
Celli's Bouillon Fucus Medium..... 2382

2372. Marpman's Chondrin Medium

Constituents:

1. Water.
2. Cartilage or ear muscles.

Preparation:

(1) Boil ripe pieces of cartilage or ear muscle in water under an atmosphere pressure in an autoclave, or in small amounts in a pressure flask which stand in 100.0% solution of Na_2SO_4.

(2) Filter thru a filter paper.

Sterilization: Not specified.

Use: Substitute for gelatin.

Reference: Marpman (1891 p. 123).

2373. Lehmann's Caragheen Medium

Constituents:

1. Distilled water..............	100.0 cc.
2. "Caragheen"................	5.0 g.

Preparation:

(1) Boil 5.0 g. of "caragheen" in 100.0 cc. of distilled water for about 30 to 60 minutes. Stir.

(2) Cool and make up the loss of water by adding distilled water.

(3) Heat to boiling again and filter thru cotton or a usual filter in the steamer.

(4) In order to hasten filtration, a 1 or 2.0% extract may be prepared and then concentrated to the desired strength after filtration.

Sterilization: Not specified.

Use: Agar substitute for solidification of liquid media. "Caragheen" is a derivative of *Chrondrus crispus.* 5.0% "caragheen" without any addition of nutrients supported the growth of parasitic and saprophytic fungi and air forms.

Reference: Lehmann (1919 p. 426).

2374. Giltner's Butter Medium

Constituents:

1. Butter.

Preparation:

(1) Melt butter fat at about 100°C.

(2) Allow the casein to settle.

(3) Decant the clear fat.

(4) Place in about 8.0 cc. sterile test tubes.

Sterilization: Sterilize by the intermittent method.

Use: To study fat decomposition. Author reported that other kinds of fat might be similarly treated.

Reference: Giltner (1921 p. 365).

2375. Mortensen's Gum Arabic Media

Mortensen's solidified media 207, 329 and 560 with gum arabic (method not given.)

2376. Schlüter's Basic Isinglass Medium

Constituents:

1. Water.......................	250.0 cc.
2. Isinglass	
3. Peptone (siccum)..........	1.25 g.
4. NaCl.......................	1.25 g.

Preparation:

(1) Prepare a decoction of isinglass in water by boiling. (Exact method not given). Filter.

(2) Dissolve 3 and 4 in (1).

(3) Dissolve one of the added nutrients in (2) in any desired concentration.

(4) Distribute into test tubes, plug and slant.

Sterilization: Method not specified.

Use: To study growth of bacteria on acid media.

Added nutrients:

Lactic acid
Tartaric acid
Citric acid
Acetic acid
HCl
Alum

Reference: Schlüter (1892 p. 592).

2377. Marpmann's Peptone Plant Tissue Medium

Constituents:

1. Water	700.0 cc.
2. Glycerol	40.0 g.
3. *Sphaerococcus confervoides*	30.0 g.
4. Peptone (liquid Koch)	20.0 g.

Preparation:

(1) Macerate 30 parts *Sphaerococcus confervoides* with 2 parts HCl and 1 liter water for two hours.

(2) Wash with water until the washings no longer turn blue litmus paper red.

(3) Add to the residue of (2) 700 parts water, 40 parts glycerol, 20 parts peptone (liquid Koch) and 2 parts beaten egg-white.

(4) Boil for 20 minutes in a steam cylinder.

(5) Neutralize.

(6) Filter thru a syrup filter, prepared from a balloon flask with a filter layer of buck-skin and felt.

Sterilization: Not specified.

Use: General culture medium. Agar substitute.

Reference: Marpmann (1891 p. 123).

2378. Matzuschita's Peptone Konbu Medium

Constituents:

1. Water	1000.0 cc.
2. Konbu	100.0 g.
3. Peptone, Koch	10.0 g.
4. Glucose	20.0 g.

Preparation:

(1) Cut 100.0 g. of dry Konbu in small pieces and mix with one liter of water.

(2) Allow to stay in the water for 24 hours in the cold.

(3) Boil for one hour in the steamer.

(4) Filter.

(5) Add 3 and 4 to the filtrate.

(6) Boil.

(7) Neutralize (indicator not specified).

Sterilization: Sterilize in the steamer on from 2 to 5 successive days for 15 to 30 minutes. Incubate for 2 days at 37°C. to test sterility.

Use: Cultivation of spore forming bacilli, *Clostridium butyricum, Bacillus oedmatis maligni, Bacillus anthracis symptomatici, Bacillus sporogenes, Bacillus botulinus.* The Konbu is a Laminaria like Japanese sea weed.

Reference: Matzuschita (1902 p. 287).

2379. Puccinelli's Infusion Fucus Medium

Constituents:

1. Meat infusion	200.0 g.
2. *Fucus crispus*	6.0 g.

Preparation:

(1) Method of preparation of meat infusion not given. Neutralize.

(2) Wash well, 6.0 g. of *Fucus crispus* in water.

(3) Boil (2) for one hour in (1) in a water bath or steamer.

(4) Filter thru a funnel heated by a flame or water funnel.

(5) Distribute in tubes and sterilize.

Sterilization: Method not given.

Use: General culture medium.

Reference: Puccinelli (1890 p. 405).

2380. Jacobi's Peptone Fucus Medium

Constituents:

1. Water	1500.0 cc.
2. Peptone (Kemmerich's)	7.5 g.
3. Peptone (siccum)	15.0 g.
4. Fucus	37.5 g.

Preparation:

(1) Dissolve 2, 3 and 4 in 1 by heating over a free flame.

(2) Make up to the original volume by addition of water.

(3) Press thru a towel, for some of the fucus will not dissolve.

(4) Add Na_2CO_3 or sodium phosphate until the reaction is slightly alkaline.

(5) Pour into a flask and steam until the albuminous material still to be removed, is completely separated.

(Usually two hours if sodium phosphate is used and longer if Na_2CO_3.)
(6) Filter thru cotton, using compressed air to effect a fast filtration.
(7) Distribute into small sterile flasks.
(8) Finally distribute sterile (7) into sterile test tubes that have been autoclaved for 2½ hours. (The tubes are contained in an enamel container in the autoclave. The inner temperature reaching about 150°C.)

Sterilization: Heat in streaming vapor for two hours.

Use: General culture medium.

Reference: Jacobi (1888 p. 540).

2381. Jacobi's Infusion Broth Fucus Medium

Constituents:

1.	Meat infusion	1500.0 cc.
2.	Peptone (siccum)	15.0 g.
3.	NaCl	7.5 g.
4.	Fucus	37.5 g.

Preparation:
(1) Method of preparation of meat infusion not given.
(2) Add 2, 3 and 4 to (1).
(3) Heat over a free flame until the materials are dissolved.
(4) Make up to the original volume by addition of water.
(5) Press thru a towel for some of the Fucus will not dissolve.
(6) Add Na_2CO_3 or sodium phosphate until the reaction is slightly alkaline.
(7) Pour into a flask and steam until the albuminous material still to be removed is completely separated. (Usually 2 hours if sodium phosphate is used and longer if Na_2CO_3.)
(8) Filter thru cotton, using compressed air to effect a fast filtration.
(9) Distribute into smaller sterile flasks.
(10) Finally distribute sterile (9) into sterile test tubes that have been autoclaved in the autoclave for 2½ hours. (The tubes are contained in an enamel container in the autoclave. The inner temperature reaching about 150°C.)

Sterilization: Heat (9) in streaming vapor for two hours.

Use: General culture medium.

Reference: Jacobi (1888 p. 540).

2382. Celli's Bouillon Fucus Medium

Constituents:

1.	Bouillon	1000.0 cc.
2.	*Fucus crispus* (5.0%)	50.0 g.

Preparation:
(1) Prepare a 5.0% solution of *Fucus crispus* in bouillon. (Author also used water instead of bouillon.)
(2) Add for each 10.0 cc. medium, 1.0 cc. of N/10 NaOH solution or 4 to 5.0 cc. saturated solution of Na_2CO_3.
(3) Distribute in plates.

Sterilization: Method not given.

Use: Isolation and cultivation of amoeba.

Reference: Celli (1896 p. 537).

GROUP V. INITIALLY LIQUID MEDIA BECOMING PERMANENTLY SOLID. SOLIDIFYING AGENT ORGANIC

Key to the Subgroups of Group V

A_1. Solidifying agent of plant origin.
Subgroup V-A (Med. 2383 to 2397)
A_2. Solidifying agent of animal origin.
B_1. Solidified by blood or its derivatives.
Subgroup V-B (Med. 2398 to 2430)
B_2. Solidified by materials other than blood or its derivatives (eggs, etc.)
Subgroup V-C (Med. 2431 to 2465)

SUBGROUP V-A

Solidifying Agent of Plant Origin Basal or Complete Media Containing Materials of Plant Origin, Initially Liquid but Becoming Permanently Solid

A_1. Additional materials, if any, of known chemical composition.
Matzuschita's Basal Rice Flour Medium.......................... 2383
Hefferan's Starch Medium.......... 2384
Lloyd, Clark and McCrae's Gluten Medium.......................... 2385
Baginsky's Basal Starch Medium... 2386
Uschinsky's Asparaginate Starch Medium (Smith).................. 2387
Hefferan's Peptone Starch Medium. 2388
Kita's Bran Medium............... 2389
Kita's Rice Medium................ 2390
Kita's Soy Bean Medium........... 2391
A_2. At least one additional constituent of unknown chemical composition.
B_1. Peptone present.
Hefferan's Peptone Rice Flour Medium.......................... 2392
Lloyd, Clark and McCrea's Bouillon Corn Flour Medium.............. 2393
Löhnis' Bouillon Milk Rice Medium. 2394
B_2. Peptone not present.
Sanguineti's Yeast Extract Starch Medium.......................... 2395
Harras' Tissue Starch Medium..... 2396
Smith's Milk Rice Medium......... 2397

2383. Matzuschita's Basal Rice Flour Medium

Constituents:
1. Water...................... 1000.0 cc.
2. Rice flour

Preparation:
(1) Boil water containing rice flour (amount not given).
(2) To the paste formed, either add 2.0% glucose or 6.0% glycerol or use plain without any additions.

Sterilization: Sterilize in the autoclave.

Use: Cultivation of mammalian and chicken tubercle bacilli. Author reported that best growth of mammalian type on glycerol medium. Its colonies were orange yellow. Chicken type gave whitish gray, grayish black or yellowish colonies.

Added nutrients: The author added 2.0% glucose or 6.0% glycerol.

Variants:
(a) The author used the basal medium without any additions.
(b) Buchanan cultivated *Monascus purpurens* from silage on a medium prepared from a thick paste prepared from rice flour and tap water.

References: Matzuschita (1899 p. 128), Buchanan (1910 p. 103).

2384. Hefferan's Starch Medium

Constituents:
1. Water...................... 1000.0 cc.
2. Starch (10.0%).............. 100.0 g.

Preparation:
(1) Cook 10.0% starch with water.
(2) Pour into petri dishes.

Sterilization: Sterilize by the discontinuous method in the steamer.

Use: Cultivation of red pigment producing organisms, *B. prodigiosus*, *B. ruber balticus*, *B. kilienis*, *B. ruber miquel*, *B. rutilus*, *B. amyloruber*. Author reported growth. Good growth was obtained when 1.0% peptone was added.

Variants: Besson prepared a similar medium as follows:

(1) Mix 10.0 g. of potato starch in 180.0 g. of water and add 5.0 g. of precipitated chalk.
(2) Distribute in flasks or petri dishes.
(3) Sterilize at 115°C.

References: Hefferan (1903–04 p. 520), Besson (1920 p. 57).

2385. Lloyd, Clark and McCrea's Gluten Medium

Constituents:

1. Water.
2. Gluten flour.

Preparation:

(1) Mix tap water and gluten flour so that a thick, but not sticky paste is formed.
(2) Place in petri dishes.

Sterilization: Autoclave for 20 minutes at 115°C.

Use: Cultivation of Flügge's Mesentericus group.

Reference: Lloyd, Clark and McCrea (1920–21, p. 383).

2386. Baginsky's Basal Starch Medium

Constituents:

1. Water	400.0 cc.
2. Starch	20.0 g.

Preparation:

(1) Boil 20.0 g. sugar free amylum with 400.0 cc. water until a starch paste is formed.
(2) Distribute in 50.0 cc. lots in previously sterilized Erlenmeyer flasks.
(3) Add one of the added nutrients.

Sterilization: Sterilize in flowing steam.

Use: To study fermentations.

Added nutrients: The author added 1.0 g. peptone or 10.0 cc. g. a solution of 1.0 g. $(NH_4)_2SO_4$ and 1.0 g. or potassium phosphate in 100.0 cc. water to each flask.

Reference: Baginsky (1888, p. 434–462).

2387. Uschinsky's Asparaginate Starch Medium (Smith)

Constituents:

1. Water	1000.0 cc.
2. Ammonium lactate	5.0 g.
3. Sodium asparaginate	2.5 g.
4. Na_2SO_4	2.5 g.
5. NaCl	2.5 g.
6. K_2HPO_4	2.5 g.
7. $CuCl_2$	0.01 g.
8. $MgSO_4$	0.01 g.
9. Starch (potato)	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.
(2) Dry potato starch.
(3) Add 10.0 cc. of (1) to each test tube containing 3.0 g. of (2).
(4) Stir the mixture with a glass rod until the starch has dissolved.
(5) Place the tubes in a slanted position at 75° to 85°C. for 20 minutes to 3 hours for 5 or 6 successive days. It may be necessary to add 2.0 cc. distilled water to each tube to make up for evaporation after the second heating. The plugs must fit well.

Sterilization: See step (5) above.

Use: To study diastase action. Author reported that other starches may be used instead of potato starch. Sugars and high alcohols may be added, 500 mg. per 10.0 cc. of medium. *B. coli commune Ps. hyacinthi*, *Sarcina aurantiaca* and others grew very well on this medium if sugars were added. Smith also used the medium to cultivate plant parasites.

Variants:

(a) Smith added 1.0 g. portions of starch to each 10.0 cc. of medium instead of 3.0 g. The starch was prepared as follows:
    (1) Scrub one-half bushel of large smooth potatoes and dig out the black spots.
    (2) Soak for 45 minutes in a 1:1000 mercuric chloride solution.
    (3) Sterilize the hands by thoroly washing in the mercuric chloride solution.
    (4) Rinse the tubers in sterile water, pare deeply and run thru a grating machine.
    (5) Throw into several liters of distilled water and work the pulp thoroly with the hands to liberate as much of the starch as possible.
    (6) Filter thru several layers of gauze.
    (7) Allow the starch to settle and decant off the brownish fluid.
    (8) Stir up the sediment in a little water and force the starch thru a moderately meshed towel with gentle rubbing.

(9) Allow the starch to stand in the ice box for about a week in distilled water (3 liters per jar or beaker). Siphon off the water twice a day at first, and afterwards once a day, stirring up the starch thoroughly each time fresh water is added.

(10) Drain the starch very free from water, scoop up with sterile spoons or spatulas and place in sterile petri dishes.

(11) Dry in the blood serum oven at 56°C., the cover being raised on corks an inch to let the moisture out.

(b) Smith prepared a similar medium as follows:

(1) Dissolve 30.0 g. to 40.0 g. glycerol, 5.0 to 7.0 g. NaCl, 0.1 g. $CaCl_2$, 0.3 to 0.4 g. $MgSO_4$, 2.0 to 2.5 g. K_2HPO_4, 6.0 to 7.0 g. ammonium lactate, 3.0 to 4.0 g. sodium asparaginate in 1000.0 cc. of water. The glycerol may be omitted.

(2) Add 1.0 g. of potato starch to each 10.0 cc. of (1) in a test tube, and proceed as above.

References: Smith (1899 p. 102), (1905 p. 196), Tanner (1919 p. 61).

2388. Hefferan's Peptone Starch Medium

Same as medium 26 with the addition of 1.0% peptone.

2389. Kita's Bran Medium

Constituents:
1. Water.
2. Wheat bran.

Preparation:

(1) Add 25.0 g. wheat bran to 10.0 g. water or 10.0% wheat bran to 20.0 g. water. Details of preparation not given.

(2) Place in Erlenmeyer flasks.

Sterilization: Not specified.

Use: Cultivation of Japanese molds, *Aspergillus Okazaki*, *Aspergillus candidus*, *Aspergillus albus*, *Aspergillus tamarii*, Pseudorhizopus, *Aspergillus glaucus* and other molds.

Variants:

(a) The author used rice bran instead of wheat bran.

(b) The author steamed 10.0 g. of rice bran with 10.0 g. of water in a 400.0 cc. Erlenmeyer flask.

Reference: Kita (1913 p. 446), (1914 p. 354).

2390. Kita's Rice Medium

Constituents:
1. Water.
2. Rice.

Preparation:

(1) Prepare one of the following combinations:

(a) 100.0 g. rice + 100.0 g. water.
(b) 25.0 g. rice + 10.0 cc. water.
(c) 10.0 g. rice + 5.0 cc. water.
(d) 100.0 g. rice + 50.0 cc. water.

(2) Place in Erlenmeyer flasks.

Sterilization: Not specified.

Use: Cultivation of Japanese molds, *Aspergillus Okazaki*, *Aspergillus candidus*, *Aspergillus albus*, *Aspergillus tamarii* Pseudorhizopus *Aspergillus glaucus* and other molds.

Variants: The author steamed 25.0 g. of rice with 15.0 cc. of water in a 400.0 cc. Erlenmeyer flask.

Reference: Kita (1913 p. 446), (1914 p. 353).

2391. Kita's Soy Bean Medium

Same as medium 2390 but the author used fat-free soy bean flour instead of rice.

Reference: Kita (1914 p. 356), (1913 p. 446).

2392. Hefferan's Peptone Rice Flour Medium

Constituents:

1. Water	100.0 cc.
2. Rice flour	35.0 g.
3. Peptone	1.0 g.

Preparation:

(1) Cook 35.0% rice flour and 1.0% peptone with water.

(2) Pour into petri dishes.

Sterilization: Sterilize by the discontinuous method.

Use: Cultivation of red pigment producing bacteria *B. prodigiosus*, *B. ruber balticus*, *B. kilienis*, *B. ruber miquel*, *B. rutilus*, *B. amyloruber*.

Reference: Hefferan (1903–04 p. 520).

2393. Lloyd, Clark and McCrea's Bouillon Corn Flour Medium

Constituents:

1. Bouillon	1000.0 cc.
2. Corn flour starch	70.0 g.

Preparation:
(1) Exact composition of broth not given.
(2) Neutralize (1) using phenol red as an indicator.
(3) Add starch to (2).
(4) Shake well.
(5) Tube.
(6) Gelatinize by boiling, shaking until gelatinization is complete.

Sterilization: Sterilize in the autoclave at 20 minutes at 115°C.

Use: Cultivation of Flügge's Mesentericus group. Author especially recommended this medium for stab cultures.

Reference: Lloyd, Clark and McCrea (1920–21 p. 383).

2394. Löhnis' Bouillon Milk Rice Medium

Constituents:

1.	Bouillon	50.0 cc.
2.	Rice meal	100.0 g.
3.	Milk	150.0 cc.

Preparation: (1) Mix 1, 2 and 3.

Sterilization: Sterilize on each of three successive days.

Use: Cultivation of molds.

Variants:
(a) Besson prepared the medium as follows:
  (1) Thoroughly mix 100.0 g. of rice powder with 50.0 cc. of peptone bouillon and 150.0 cc. of milk.
  (2) Distribute in layers 1 to 2 centimeters thick in petri dishes.
  (3) Heat at 115°C. for 20 minutes.
  Besson noted that this medium had been described by Soyka.
(b) Harvey prepared a medium as follows:
  (1) Prepare a paste from 10.0 parts rice flour, 7 parts infusion broth (see 779 (bb)) and 2 parts milk.
  (2) Distribute as a layer in test tubes or flasks.
  (3) Heat over boiling water to solidify the paste.
  (4) Sterilize 30 minutes at 100°C. on 3 days.
(c) Harvey prepared a similar medium as follows:
  (1) Prepare a paste from 2 parts rice flour, 1 part infusion broth (see 779 (bb)) and 3 parts milk.
  (2) Distribute as a layer in test tubes or flasks.
  (3) Sterilize 30 minutes at 100°C. on 3 days.
  (4) The paste may be distributed in petri dishes and sterilized at 115°C. for 20 minutes.

References: Löhnis (1913 p. 88), Besson (1920 p. 58), Tanner (1919 p. 72), Harvey (1921–22 p. 95).

2395. Sanguineti's Yeast Extract Starch Medium

Constituents:

1.	Yeast extract	500.0 cc.
2.	Starch	15.0 g.

Preparation:
(1) Preparation of yeast extract not given.
(2) Distribute yeast extract in 500.0 cc. lots in 1500.0 cc. flasks.
(3) Add 15.0 g. of starch to each flask.
(4) Boil for 30 minutes on a salt water bath.

Sterilization: Autoclave for 30 minutes at 120°C.

Use: To study fermentation of starch by *Aspergillus orizae*, *Mucor alternans*, *Amylomyces Rouxii*.

Reference: Sanguineti (1897 p. 267).

2396. Harrass' Tissue Starch Medium

Constituents:
1. Water.
2. Liver or brain.
3. Starch.

Preparation:
(1) Pass liver or brain through a meat grinder.
(2) Prepare a paste with starch in the usual manner. (Method not given.)
(3) Mix (1) and (2) thoroughly (amounts not specified).
(4) Distribute in petri dishes.

Sterilization: Sterilize in streaming steam.

Use: Cultivate obligate anaerobes. *Bac. butyricus*, *Bac. botulinus*, the black leg bacillus, the maligenens oedema bacillus, and other obligate anaerobes. This medium gave a quite solid medium enabling one to obtain pure cultures.

Reference: Harrass (1906 p. 2339).

2397. Smith's Milk Rice Medium

Constituents:

1. Milk.................. 100.0 cc.
2. Rice.................. 10.0 to 20.0 g.

Preparation: (1) Place one or two grams of rice into each tube containing 10.0 cc. of milk.

Sterilization: Not specified.

Use: Cultivation of chromogenic plant parasites. Klimmer cultivated skin microphytes on a similar medium.

Variants: Klimmer prepared a similar medium as follows:

(1) Thoroughly mix 100.0 g. of rice powder with 250.0 cc. of skimmed cow's milk.
(2) Heat in a porcelain dish, stirring constantly.
(3) Pour into cylindrical tubes.
(4) When cool, shove the solidified rice milk mixture from the tubes and cut into dices.
(5) Place the discs into petri dishes.
(6) Add 8 drops of milk to each disc.
(7) Sterilize for 1 to 1.5 hours.

Klimmer gave Kral the credit for describing this medium.

References: Smith (1905 p. 48), Klimmer (1923 p. 207).

SUBGROUP V-B

Basal or Complete Media Solidified by Blood or Derivatives, Initially Liquid but Becoming Permanently Solid

A_1. Additional constituents, if any, of known chemical composition.

B_1. Additional organic constituents not employed.

Frothingham's Coagulated Blood Serum.......................... 2398
Proco, et al., Gentian Violet Serum. 2399
Lorrain Smith's Alkali Serum (Harvey)...................... 2400
Duval's Trypsinized Serum......... 2401
Carrel and Burrows' Plasma Medium.......................... 2402
Ball's Coagulated Blood Medium... 2403

B_2. Additional organic constituents employed.

C_1. Carbohydrates present.

Cobbetts' Glucose Serum Medium.. 2404
Costa's et al. Litmus Glucose Serum.......................... 2405
Rankin's Potassium-Sulphocyanide Glucose Serum.................. 2406
Dubois' Glycerol Glucose Serum.... 2407

C_2. Carbohydrates not present.

Marx's Glycerol Ragit Serum....... 2408
Ficker's Glycerol Phosphate Serum. 2409
Ficker's Citrate Glycerol Serum.... 2410
Proca, et al. Pyrogallic Acid Serum. 2411
Shmamine's Nucleate Serum........ 2412

A_2. At least one additional constituent of unknown chemical composition.

B_1. Carbohydrates added.

C_1. Additional organic carbon of known chemical composition added.

Conradi and Troch's Extract Broth Serum Medium.................. 2413
Hall and Stone's Glycerol Serum Medium.......................... 2414
Greenspon's Veal Infusion Serum (Stitt).......................... 2415
Bacto Loeffler's Blood Serum (Dehydrated).................... 2416
Loeffler's Glucose Infusion Serum.. 2417
Rankin's Cyanide Glucose Serum... 2418
Ball's Glucose Bouillon Blood Albumin Medium.................. 2419
Crowe's Glucose Agar Blood Medium.......................... 2420

C_2. Additional organic carbon of known chemical composition not supplied.

B_2. Carbohydrates not added.

Yardon's Bouillon Serum (Desiccated)......................... 2421
Thalmann's Infusion Broth Serum Medium.......................... 2422
Ficker's Brain Infusion Serum Medium.......................... 2423
Ficker's Potato Juice Serum Medium.......................... 2424
Vannod's Nutrose Serum Medium.. 2425
Pergola's Egg Yolk Serum Medium. 2426
Greenspon's Glucose Citrate Serum Medium.......................... 2427
Duval's Egg Serum Medium........ 2428
Eberling's Serum Tissue Plasma Medium.......................... 2429
Wolbach and Schesinger's Tissue Plasma Medium.................. 2430

2398. Frothingham's Coagulated Blood Serum

Constituents: 1. Serum

Preparation:

(1) Collect blood in large jars which can be closed tightly. Close the jars.

(2) When the blood has begun to clot, pass a sterile glass rod around the clot between its surface and the wall of the jar. This permits the clot to sink.

(3) Close the jars and place in an ice box for 24 to 48 hours.

(4) Draw off the serum with a sterile pipette and distribute into sterile test tubes.

(5) Place in a blood serum sterilizer and heat to 68 or 70°C. for one hour on five successive days.

(6) Place the tubes in the apparatus for solidifying blood serum and heat to 75 or 80°C. until the serum is coagulated.

Sterilization: Sterilization given with method of preparation.

Use: Culture medium for parsitic forms.

Variants: The following methods of preparation have been given by the following investigators:

(a) Frothingham heated the serum collected as indicated above, at 90 to 95°C. for an hour or more, and then steamed on each of 2 successive days for 15 to 30 minutes.

(b) Michel cultivated diphtheria bacilli on the following medium:

(1) Obtain horse or beef serum under sterile conditions.

(2) Distribute into sterile test tubes.

(3) Solidify in a Koch's apparatus at a temperature of 85°C.

(c) Wurtz.

(1) Collect blood from the slaughter house under aseptic conditions in petri dishes, Erlenmeyer flasks or jars.

(2) When the blood has clotted pipette off the serum by means of a sterile pipette.

(3) Heat for 3 hours at 58°C. on each of three successive days to sterilize (Tyndall method).

(4) Distribute as desired.

(5) Coagulate by heating between 90 and 100°C.

(d) Glücksmann cultivated diphtheria bacilli on a medium prepared as follows:

(1) Collect beef blood under aseptic conditions from a slaughter house into a sterile four liter cylinder.

(2) Allow to stand in the ice box for 4 or 5 days at 7° to 8°C.

(3) Pipette the serum under aseptic conditions into sterile test tubes in 4.0 cc. lots.

(4) Solidify by heating in a slanted position at a temperature of 70 to 72°C. for about one hour.

(5) Incubate 2 or 3 days to test sterility.

(e) Smith cultivated tubercle bacilli on the following medium:

(1) Bleed a dog under chloroform and draw the blood from a femoral artery under aseptic conditions thru sterile tubes directly, or into sterile flasks.

(2) Draw the serum from the clots with sterile pipettes and either distribute at once into sterile test tubes or else store with 0.25 to 0.3% chloroform.

(3) Slant the tubes and heat to 75° to 76°C. to coagulate, requiring about three hours. No other sterilization or heating is required. If chloroform has been added it is necessary to place the tubes for an hour or longer in a water bath at 55–60°C. or under the receiver of an air-pump to drive off the antiseptic.

(4) Keep in cold closed space and in an inclined position before inoculation.

(5) Use a ground glass stopper instead of the ordinary cotton plug to close the tubes.

(f) Thoinot and Masselin.

(1) Obtain blood under as nearly aseptic conditions as possible in large glass jars.

(2) Store in a cool place for 24 to 48 hours to allow the blood to clot.

(3) Remove the serum from the clot by means of a sterile pipette.

(4) Distribute into sterile tubes.

- (5) Solidify in a Koch inspissator at 68 to 70°C.

(g) Heinemann.

- (1) Coagulate dog's blood serum in slanted tubes in the Koch inspissator for three hours at 75°C.

(h) Schereschewsky cultivated *Syphilis spirochaete* on the following medium:

- (1) Draw off normal horse serum, by means of a syphon under aseptic conditions into thin walled sterile tubes about 10 to 15 cm. long. Allow the serum to run down the wall of the tube so as not to mix the serum with air.
- (2) Seal the tubes with cotton or cork and gum.
- (3) Heat in a water bath at 57–58°C. for one hour.
- (4) Raise the temperature slowly to 70°C.
- (5) When the serum has solidified, incubate at 37°C. over night to test sterility.

(i) Sowade used the following medium to cultivate syphilis spirochaete:

- (1) Collect horse blood in long measuring cylinders.
- (2) Place in the ice box until the serum separates.
- (3) Distribute into test tubes to a depth of 12.0 cm.
- (4) Heat in a water bath on 3 successive days for two hours at 58°C.
- (5) On the third day slowly raise the temperature until the serum reaches a gelatinous consistency. Raise the temperature slowly or an intensive turbidity will take place.
- (6) Medium is honey yellow, transparent and reacts slightly alkaline.

(j) Abbott.

- (1) Obtain blood serum from the slaughter house or anti-toxin manufacturers.
- (2) Tube in sterile tubes and plug.
- (3) Slant.
- (4) Place in a dry air sterilizer and slowly raise the heat to 80–90°C. Keep at this temperature until the serum has solidified.
- (5) Steam on three successive days for 20 minutes at 100°C.

(k) Ball.

- (1) Collect blood under aseptic conditions at the slaughter house, if possible, in large tall sterile flasks.
- (2) Place on ice for 48 hours.
- (3) Draw out the serum by means of sterile pipettes into test tubes. Do not shake the jar.
- (4) Place the tubes in an inspissator in a slanted position.
- (5) Heat to 65° to 68° until coagulation occurs.
- (6) Remove the tubes and sterilize by the fractional method.
- (7) Keep for 3 or 4 days in the incubator at 58°C. and discard those tubes showing growth. These tubes are transparent and straw colored.
- (8) The serum may be prepared by coagulating at a temperature short of the boiling point—temperature not specified. Sterilize by exposing the tubes to a temperature of about 90°C. on each of three successive days for 5 minutes. These tubes are opaque and white.

(l) Harvey.

- (1) Collect ox or sheep blood at the slaughter house in a sterile blood jar.
- (2) Allow the blood to coagulate.
- (3) Detach the clot.
- (4) Place in the ice chest.
- (5) Pipette off the serum with a sterile pipette.
- (6) Keep the serum in the ice chest till required.
- (7) Distribute a portion of the serum in quantities of 5.0 cc. in test tubes.
- (8) Sterilize 2 days 30 minutes at 60°C.
- (9) Coagulate in a slanting position in an inspissator, or over steam at temperatures varying from 65 to 90°C. according to the degree of transparency required.

(m) Harvey.

- (1) Collect ox or sheep blood at the slaughter house in a sterile blood jar or in a bucket.
- (2) Allow the blood to coagulate before removing it.
- (3) Separate the clot from the sides of the containing vessel with a sterile glass rod.

(4) Place in a cool place for 24 hours.
(5) Transfer the separated serum with a sterile pipette in quantities of 5.0 cc. to sterile test tubes.
(6) Sterilize 30 minutes at 58°C. on each of 8 successive days or 20 minutes at 100°C. three days.
(7) Test sterility by incubation for 48 hours.

(n) Harvey.
(1) Prepare: chloroform 2; blood serum 100.
(2) Keep in a well stoppered bottle in the dark.
(3) Distribute in sterile test tubes with sterile precautions.
(4) Coagulate in a slanting position in an inspissator or over steam at temperatures varying from 65 to 90°C. according to the degree of transparency required.

(o) Harvey.
(1) Coagulate serum in a slanting position in an inspissator or over steam at temperatures varying from 65 to 90°C. according to the degree of transparency required.
(2) Add 0.85 sterile salt solution to each test tube, slope to cover the medium.
(3) Sterilize 60 minutes at 115°C.
(4) Pour off the salt solution at the time of use.

NOTE: The addition of salt solution allows for satisfactory sterilization and keeps the medium moist till required.

(p) Harvey cultivated spirochaetes on the following medium:
(1) Fill the serum into tall test tubes.
(2) Heat in the upright position at 65°C.
(3) Remove as soon as the serum begins to set.

NOTE: The heat retained in the tube will complete the coagulation. A soft, almost transparent coagulum is formed.

(q) Harvey prepared a medium as follows for the cultivation of spirochaetes:
(1) Prepare: horse serum 3; distilled water 1.
(2) Distribute in quantity to nearly fill test tubes.
(3) Close the test tube with a rubber cork.
(4) Heat one hour at 60°C. in a water bath.
(5) Heat, 24 hours later one hour at 70°C. in a water bath.
(6) Heat, 24 hours later at 70°C. until the medium becomes syrupy.
(7) Keep in the ice chest till required for use.

(r) Pitfield.
(1) Obtain dog, sheep or cow blood under aseptic conditions.
(2) Pipette off the serum with a sterile pipette.
(3) Distribute into sterile test tubes.
(4) Coagulate by heat.

(s) Jones cultivated an organism resembling *Bacillus actinoides* from pneumonic rat lungs on a medium prepared as follows:
(1) Coagulate horse serum in tubes.
(2) Remove a small portion of the lung of an infected rat under sterile precautions.
(3) Add 0.5 cc. of sterile calf serum water to the water of condensation.
(4) Push the piece of tissue down the tube and into the liquid at the bottom.
(5) Seal the tubes with sealing wax.

(t) Vardon prepared a desiccated medium as follows: He reported that the dried serum when dissolved in water and brought to its original volume can be used for all purposes where serum is used in media.
(1) Measure out a quantity of serum.
(2) Pour into shallow trays.
(3) Dry at a temperature not exceeding 50°C.

NOTE: Coagulation of the serum should not occur in the process of drying. The drying process may be continued overnight by placing the trays in a large incubator.

To use powder.
(4) Dissolve by shaking the requisite amount, calculated from amount of dry material obtained from the liquid, of (3) in cold water.
(5) Distribute when dissolved in amounts of about 7.0 cc. into test tubes.

(6) Inspissate the medium in a sloping position at a temperature of 75°C. for 60 minutes on three successive days.
(7) Incubate tubes 24 hours and reject any which are not sterile.

(u) Park, Williams and Krumwiede cultivated spirochaetes on a medium prepared as follows:
(1) Tube serum in tall tubes.
(2) Warm the tubes to 65°C.
(3) Place a few of the tubes at a time in water at 75°C.
(4) Tilt these tubes every few seconds and remove as soon as they start to set. The heat in the tube completes the coagulation.

They reported that the serum may be diluted with saline or broth and treated in the same way.

References: Frothingham (1895 pp. 55, 57), Marmier (1895 p. 569), Michel (1896 p. 261), Wurtz (1897 p. 33), Glücksmann (1897 p. 436), Smith (1898 p. 456), Thoinot and Masselin (1902 p. 42), Heinemann (1905 p. 131), Schereschewsky (1912 p. 1335), Sowade (1912 p. 797), Abbott (1921 p. 135), Ball (1919 p. 78), Harvey (1921–22 pp. 78–81), Pitfield (1922 p. 119), Jones (1922 p. 363), Vardon (1923–24 p. 431), Park, Williams and Krumwiede (1924 p. 125).

2399. Proco, et al., Gentian Violet Serum

Constituents:
1. Water.
2. Serum.
3. Gentian violet.
4. NaCl.

Preparation:
(1) Dissolve 0.1 centigrams of gentian violet in 300.0 cc. of physiological salt solution.
(2) Mix equal parts of tyndallized serum and (1).
(3) Coagulate at 80°C.

Sterilization: Sterilization given in the preparation.

Use: Cultivation of *Syphilus spirochaete*

References: Proco, Danila and Stroe (1912 p. 896), Harvey (1921–22 p. 81).

2400. Lorrain Smith's Alkali Serum (Harvey)

Constituents:

1. 10.0% NaOH	1.5 cc.
2. Ox serum	100.0 cc.

Preparation:
(1) Add 15.0 cc. of 10.0% NaOH to 1000.0 cc. of ox serum.
(2) Coagulate in an inspissator or over steam at 65 to 90°C. according to the degree of transparency required.

Use: General culture medium.

Reference: Harvey (1921–22 p. 78).

2401. Duval's Trypsinized Serum

Constituents:
1. Serum (human).

Preparation:
(1) Pour human blood serum into sterile petri dishes and inspissate for three hours at 70°C. (Egg albumin or nutrient agar with or without 1.0% tryptophane may be treated in the same manner.)
(2) Cut an excised leprous nodule into thin slices, two to four millimeters in breadth and 0.5 to 1.0 millimeters in thickness and distribute over the surface of the coagulated serum (albumin or agar).
(3) The medium is now seeded. Bathe the surface with a 1.0% sterile trypsin solution, taking care not to submerge the piece of leprous tissue. Add sufficient trypsin solution to completely cover the medium.
(4) Incubate in a moist chamber at 37°C. for a week or ten days. Remove the plates from time to time and add more trypsin as is necessary.

Sterilization: Method not given.

Use: Isolation and cultivation of *B. leprae*. The author reported that the colonies at first were grayish white, but after several days they assumed a distinct orange yellow tint. Transfers may be made from this medium to slants or other plates.

Reference: Duval (1911 p. 369).

2402. Carrel and Burrows' Plasma Medium

Constituents: 1. Blood plasma

Preparation:

(1) Obtain the blood from the animal whose tissue are to be cultivated or another animal of the same species, under aseptic conditions. Collect the blood in sterile tubes cooled to 0°C., coated with sterile paraffin.

(2) Cork the tubes immediately and place in large tubes filled with ice and centrifuge for 5 minutes, and place in a small ice box at 0°C.

(3) Remove the supernatant plasma with sterile paraffin coated pipettes. It is generally used immediately.

(4) Take the tissue to be cultivated directly from the living animal or immediately after death. Cut the tissue in small pieces and transfer either to a cover glass or larger container. The cutting must be done quickly for desiccation kills the tissue.

(5) Cover the tissue with the plasma.

Sterilization: See preparation.

Use: Cultivation of tissue. Author reported that citrated blood might be used. Sufficient blood was added to a 1.0% sodium oxalate solution making the solution 0.1%. At the time of use precipitate the sodium oxalate quantitively from the plasma by the addition of $CaCl_2$. This does not give as good results as does pure plasma.

Variants: Carrel prepared the medium as follows:

(1) Dilute plasma with about 2/5 of its volume of dist. water.

(2) In order to accelerate the coagulation of the plasma, embryonal extracts or serum may be added.

(3) Pour (1) over small pieces of tissue to be cultivated.

Reference: Carrel and Burrows (1911 p. 391), Carrel (1912 p. 393).

2403. Ball's Coagulated Blood Medium

Constituents: 1. Blood.

Preparation: (1) Coagulate blood (not the serum only) in test tubes.

Sterilization: Not specified.

Use: General culture medium.

Reference: Ball (1919 p. 80).

2404. Cobbett's Glucose Serum Medium

Constituents:

1. Serum	1000.0 cc.
2. Glucose	20.0 g.

Preparation:

(1) Collect beef blood from a slaughter house and store the blood in a cool place to allow the serum to separate.

(2) To 100.0 cc. of serum add 2.0 g. of glucose and 1.75 cc. of 10% NaOH solution.

(3) Distribute into test tubes (or Petri dishes).

(4) Slant the tubes and place in the autoclave. In order to avoid air bubbles in the medium close the stop cock of the autoclave before all the air is removed. This gives a high pressure.

Sterilization: Sterilize for 20 minutes at 120°C.

Use: Diagnosis of diphtheria. Author reported that diptheria colonies were flat, gray or colorless. After a few days surface became irregular. Different sera may require different amounts of alkali. Old laboratory strains grew less luxuriantly than did freshly isolated strains.

Variants: The author prepared a similar medium as follows:

(1) To 100.0 cc. of horse serum add 2.0 g. dextrose, and 1.25 to 1.3 cc. of 10.0% NaOH solution.

(2) Distribute into tubes or Petri dishes.

(3) Heat to 90°C. on two successive days to sterilize.

Reference: Cobbett (1898 p. 395), (1898 p. 362).

2405. Costa's et al. Litmus Glucose Serum

Constituents:

1. Serum (horse)	100.0 cc.
2. Glucose	
3. Litmus	
4. H_2SO_4	

Preparation:

(1) Prepare a solution of 30 parts glucose to 100 of water.

(2) Mix 10.0 cc. of sterile (1), 30 drops of a sterile concentrated solution of tincture of litmus, 3.0 cc. of a sterile one to one hundred H_2SO_4 and 100.0 cc. of sterile horse serum.

(3) Coagulate in the autoclave for 75 minutes at 75 to 80° or in a hot air oven at the same temperature and time.

Sterilization: Method of sterilization of glucose, litmus or H_2SO_4 solutions not given.

Use: Diagnosis of diphtheria. Author reported that diphtheria colonies were red

Variants: Harvey prepared the litmus tincture as follows:

(1) Grind up litmus in a mortar.
(2) Add 5 volumes 90 per cent alcohol.
(3) Boil on a water bath.
(4) Decant the supernatant fluid.
(5) Add 6 parts distilled water to the residue.
(6) Boil.
(7) Allow to cool.
(8) Divide into two portions.
(9) Render one portion slightly red with dilute sulphuric acid.
(10) Add to this reddened portion the other untreated portion little by little until the mixture becomes blue again.
(11) Filter thru paper when cool.
(12) Distribute into test tubes.
(13) Sterilize at 110°C.
(14) Keep for use.

References: Costa, Troisier and Dauvergne (1917 p. 80), Harvey (1921–22 p. 81).

2406. Rankin's Potassium-Sulphocyanide Glucose Serum

Constituents:

1. Water	100.0 cc.
2. Sheep serum	300.0 cc.
3. Glucose	4.0 g.
4. 1.0% aqueous neutral red solution	4.0 cc.
5. 50.0% potassium sulphocyanide solution	8.0 cc.

Preparation:

(1) Obtain fresh serum from sheep blood. Serum should contain no red blood cells.
(2) Add 100.0 cc. of water to 300.0 cc. of (1). (Author used bouillon or equal parts of water and bouillon and water, but water gave equally good results.)
(3) Add 1.0% glucose, 4.0 cc. of a 1.0% neutral red solution and 8.0 cc. of a 50.0% solution of potassium-sulphocyanide solution.

Sterilization: Tube and sterilize in the steamer for an hour on three successive days at a temperature of from 180 to 190°F.

Use: Show presence of *B. diphtheriae* in throats. Author reported that *B. diphtheriae* always gave a characteristic red colony. He recommended that the medium be used in the discharging of patients.

Reference: Rankin (1912 p. 63).

2407. Dubois' Glycerol Glucose Serum

Constituents:

1. Serum	100.0 cc.
2. Glucose	7.0 g.
3. Glycerol	2.0 g.

Preparation:

(1) Tube non-sterilized serum containing 7 parts glucose and 2 parts glycerol per 100 in 2.0 cc. quantities.
(2) Solidification not specified.

Sterilization: Not specified.

Use: Cultivation of Koch's bacilli.

Reference: Dubois (de Nancy) (1896 p. 204).

2408. Marx's Glycerol Ragit Serum

Constituents:

1. Water	1000.0 cc.
2. "Ragit" serum	130.0 g.
3. Glycerol	50.0 cc.

Preparation:

(1) Pour 13.3 g. "Ragit" serum in a large mortar.
(2) Add 100.0 cc. water in small lots.
(3) When the serum has become entangled by mixing with a pestil, add a few drops of water and rub the water in the powder.
(4) As soon as the mixture has become thick add more water. Always rub in a circle as if mixing milk and flour in cooking. Continue this until all the 100.0 g. has been added.
(5) Add 5.0 cc. of glycerol to the mixture of 13.3 g. "Ragit" serum and water.
(6) Distribute into tubes or plates.
(7) Solidify by placing the tubes or plates in flowing steam.

Sterilization: Place the medium in the autoclave for 15 minutes (pressure not specified).

Use: Cultivation of diphtheria bacilli. Author reported that growth was nearly as good on this medium as on Loeffler's serum.

Reference: Marx (1913 p. 250).

2409. Ficker's Glycerol Phosphate Serum

Constituents:

1. Blood serum	1000.0 cc
2. KH_2PO_4	5.0 g.
3. Glycerol	20.0 g.

Preparation:

(1) Add 0.5% KH_2PO_4 to blood serum.
(2) Add 2.0% glycerol to (1).
(3) Solidify (method not given).

Sterilization: Method not given.

Use: Cultivation of tubercle bacilli. Author reported that the tubercle bacilli grew without the glycerol. In concentration higher than 2.0% glycerol checked the growth.

Variants:

(a) Thoinot and Masselin prepared a similar medium as follows:
  (1) Autoclave glycerol at 115°C.
  (2) Add 6 to 8 parts by weight of (1) to 100 parts of sterile serum.
  (3) Mix well.
  (4) Tube.
  (5) Solidify at a temperature between 75 to 80°C.
(b) Harvey prepared a similar medium by adding 7 parts sterile glycerol to 100 parts sterile ox serum, tubing and coagulating in the steamer or inspissator at 65° to 90°C.
(c) Harvey gave the following method of preparation:
  (1) Mix 5 parts glycerol and 95 parts ox serum.
  (2) Heat 30 minutes at 56°C. in a water bath on each of two successive days.
  (3) Coagulate in the inspissator or steamer at 65° to 90°C.
  (4) Test sterility before use by incubating 48 hours.

References: Ficker (1900 p. 510), Thoinot and Masselin (1902 p. 48), Roux and Rochaix (1911 p. 128), Besson (1920 p. 53), Dopter and Sacquépeé (1921 p. 139), Harvey (1921–22 pp. 78, 79).

2410. Ficker's Citrate Glycerol Serum

Constituents:

1. Distilled water	20.0 cc.
2. Blood serum	80.0 cc.
3. Magnesium citrate	0.25 g.
4. $(NH_4)_2SO_4$	0.3 g.
5. Potassium phosphate	0.5 g.
6. $MgSO_4$	0.25 g.
7. Glycerol	2.0 g.

Preparation:

(1) Dissolve 3, 4, 5, 6 and 7 in 1.
(2) Add 80.0 cc. of serum.
(3) Solidify (method not given).

Sterilization: Method not given.

Use: Cultivation of tubercle bacilli.

Variants: The author omitted the $MgSO_4$, used 0.2 g. $(NH_4)_2SO_4$ instead of 0.3 g. and added 0.6 g. mannitol.

Reference: Ficker (1900 p. 510).

2411. Proca, et al., Pyrogallic Acid Serum

Constituents:

1. Distilled water.
2. Serum.
3. Pyrogallic acid.

Preparation:

(1) Dissolve 1.0 g. pyrogallic acid and 2.0 g. NaOH in 100.0 cc. distilled water.
(2) To 10.0 cc. serum (beef or horse) add 1.0 cc. of (1).
(3) Coagulate at 80°.

Sterilization: Sterilize the serum in the steamer using Tyndall's method.

Use: Cultivation of syphilus spirochetes.

Variants: Harvey specified that the pyrogallic acid solution be stored several weeks before use, and inoculated the solidified medium by passing a pipette containing a culture material between the medium and the wall of the tubes, seal the tubes hermetically and then incubate.

References: Proca, Danila and Stroe (1912 p. 895), Harvey (1921–22 p. 80).

2412. Shmamine's Nucleate Serum

Constituents:

1. Serum	200.0 cc.
2. Sodium salt of nucleic acid	1.0 to 1.5 g.

Preparation:

(1) Obtain horse blood under aseptic conditions if possible.
(2) Draw off the clear serum by means of sterile pipettes.
(3) Allow to stand in the ice box for two or three days.

(4) This serum should be very clear, transparent, and light yellow to pale red in color.
(5) Dissolve 1.0 to 1.5 g. sodium salt of nucleic acid (Böhrenger) in 200.0 cc. of sterile (4) by shaking.
(6) Pass carbonic acid from a Kipp apparatus thru (5) for 2 or 3 minutes. This tends to clear the medium.
(7) Distribute into tall test tubes about ⅔ full and heat in a water bath on three successive days for one hour each day at 60°C.
(8) After removal from the water bath place into ice cold water.
(9) At the time of the fourth sterilization solidify the serum by gradually raising the temperature to 70°C. You can obtain three different degrees of hardness of the serum. By heating at a low temperature one obtains a soft, clear and transparent medium. By heating at a high temperature one obtains a hard solid nearly opaque medium. Then there is a medium between the two extremes.

Sterilization: Sterilize (5) by the fractional method in the steamer. Further sterilization is obtained in step (7) under the preparation.

Use: Cultivation of spirochetes.

Variants: Futaki, et al., studied the motility of *Spirochaeta morsus muris* (cause of rat bite fever) on a medium prepared as follows:

(1) Shake 0.5 to 0.75 g. of sodium nucleate with 100.0 cc. horse serum until the nucleate has dissolved.
(2) Pass CO_2 thru the medium for 2 or 3 minutes until the medium is transparent.
(3) Heat on three successive days for about an hour at 60°C.
(4) On the 4th day heat to 65°C. for 30 minutes, when a fluid and coagulated portion is formed.

References: Shmamine (1912 p. 313), Futaki, Takaki, Taniguchi and Osumi (1917 p. 35).

2413. Conradi and Troch's Extract Broth Serum Medium

Constituents:

1. Water	1000.0 cc.
2. Meat extract	10.0 g.
3. Peptone (Witte)	20.0 g.
4. NaCl	5.0 g.
5. Calcium acid malate	6.0 g.
6. Glucose	1.0%
7. Serum (beef)	
8. Potassium tellurite (1.0% solution)	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Filter.
(3) Add 1.0% glucose.
(4) Add 1 part (3) to 3 parts fresh sterile beef serum.
(5) To 100.0 cc. of this mixture add 2.0 cc. of a 1.0% potassium tellurite solution.
(6) Place in Petri dishes with bibulous paper inside covers.
(7) Solidify at 85–90°C.

Sterilization: Sterilize by repeated heating (temperature not given).

Use: Isolation of diphtheria bacilli. Author reported that diphtheria colonies were coal black due to the reduction of tellurium.

Reference: Conradi and Troch (1912 pp. 1652–1653).

2414. Hall and Stone's Glycerol Serum Medium

Constituents:

1. Water	1000.0 cc.
2. Peptone (Witte)	10.0 g.
3. NaCl	5.0 g.
4. Glucose	10.0 g.
5. Glycerol	50.0 g.
6. Serum, blood	3000.0 cc.

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Mix one part of (1) with 3 parts of 6.
(3) Filter thru Berkefeld filter.
(4) Tube.
(5) Slant.

Sterilization: Sterilize in autoclave at 10 pounds pressure for 30 minutes.

Use: Cultivation of diphtheroid bacillus of Preisz-Nocard from equine and bovine abscesses. Author reported that several strains of *B. diphtheriae* will grow on this medium.

Reference: Hall and Stone (1916 p. 196).

2415. Greenspon's Veal Infusion Serum
(Stitt)

Constituents:

1. Glucose veal infusion broth	24.0 cc.
2. Sodium citrate (50.0% soln.)	1.0 cc.
3. Serum, pig, sheep or human	75.0 cc.

Preparation:

(1) Add 1.0 cc. of a 50.0% sodium citrate solution to 75.0 cc. of clear pig, sheep or human serum and sufficient glucose veal infusion broth, or glucose meat extract broth to bring the volume to 100.0 cc.

(2) Adjust the reaction to pH = 6.4 by the addition of 3.0% citric acid solution using di-brom-thymol blue as an indicator.

(3) Tube.

(4) Coagulate in an inspissator.

Sterilization: Sterilize by the fractional method on 3 successive days.

Use: Cultivation of diphtheria bacilli. The author reported that the citric acid inhibited the growth of organisms from the mouth.

Reference: Stitt (1923 p. 41).

2416. Bacto Loeffler's Blood Serum
(Dehydrated)

Constituents:

1. Distilled water	
2. Serum, beef blood	300.0 cc.
3. Glucose infusion broth	100.0 cc.

Preparation:

(1) Dissolve 80.0 g. of Bacto Loeffler's Blood Serum (Dehydrated) in 1000.0 cc. of distilled water at 42°C.

(2) Tube in 3 to 5.0 cc. lots.

(3) Place the tubes in a slanted position in the autoclave.

(4) Close all parts and the door of the autoclave before turning on the steam. Run the pressure to 15 pounds as quickly as possible and hold it for 10 minutes, or to 10 pounds pressure for 20 minutes, or to 5 pounds pressure for 60 minutes. Do not use over 15 pounds pressure.

Sterilization: When coagulation is complete, open the lowest part and replace the entrapped air with steam, maintaining constant pressure during the change. Sterilization at 15 pounds pressure for 20 minutes following coagulation is recommeneded.

Use: Cultivation of diphtheria bacilli.

Reference: Digestive Ferments Co. (1925 p. 11).

2417. Loeffler's Glucose Infusion Blood Serum

Constituents:

1. Serum (horse)	300.0 cc.
2. Meat infusion	100.0 cc.
3. Peptone	1.0 g.
4. NaCl	0.5 g.
5. Dextrose	1.0 g.

Preparation:

(1) Prepare meat infusion in the usual manner (exact method not given).

(2) Dissolve 1.0 g. peptone, 0.5 g. NaCl and 1.0 g. glucose in 100.0 cc. (1) making a bouillon.

(3) Obtain horse serum under sterile conditions.

(4) Mix 3 parts (3) with one part sterile (2).

(5) Solidify in Koch's apparatus, heating to 90°C.

Sterilization: Not specified.

Use: Cultivation of diphtheria bacilli.

Variants: The following authors have suggested the following methods of preparation.

(a) Michel (1896).
  (1) Prepare meat infusion in the usual manner (exact method not given).
  (2) Dissolve 1.0 g. peptone, 0.5 g. NaCl and 1.0 g. glucose in 100.0 cc. (1) making a bouillon.
  (3) Obtain horse serum under sterile conditions.
  (4) Mix 3 parts (3) with one part sterile (2).
  (5) Solidify in Koch's apparatus, heating to 90°C.

(b) Smith (1902).
  (1) Preparation of veal bouillon not given.
  (2) Add 1.0 of glucose to (1).
  (3) Mix 3 parts liquid serum with 1 part sterile (2) under aseptic conditions.
  (4) Coagulate in the inspissator.

(c) Frost (1903).
  (1) Collect beef blood in sterile container at an abattoir.

(2) Allow the blood to stand undisturbed for 15 to 30 minutes or until the clot has firmly attached itself to the sides of the vessel.
(3) Cover the vessels and remove to the laboratory.
(4) Separate the clot from the sides of the vessel by means of a sterile knife, or a glass rod. Place the vessel in the ice chest.
(5) Allow to stand for 48 hours, and pipette or siphon off the serum.
(6) Place in tall cylinders (graduates) and allow to stand 24 hours.
(7) Separate the straw colored serum from the sediment.
(8) Add 0.5 cc. chloroform and preserve in tightly stoppered flasks.
(9) Mix one part of 1.0% glucose infusion broth, with 3 parts (8).
(10) Tube in sterile test tubes (about 3 cm. deep), and place in a sloping position in an inspissator or steamer and heat to 95°C. for 1 hour on 3 successive days.

(d) Heinemann (1905).
(1) Collect fresh ox blood in sterile museum jars.
(2) Set in an ice chest until the serum has separated.
(3) Filter the serum if necessary.
(4) Take 3 parts (3) and mix thoroly with 1 part of infusion broth containing 1.5% glucose.
(5) Tube.
(6) Place in a Koch inspissator two or three rows deep. Place about 25.0 cc. of water with the tubes.
(7) Incline the inspissator to the proper angle so as to slant the serum.
(8) Heat the water in the apparatus to boiling and boil 5 minutes. Then allow to cool.
(9) Repeat (8) on the two following days.

(e) Rothe (1907).
(1) Mix 4 parts sterile beef serum with 1 part sterile sugar free nutrient bouillon (method of preparation or composition not given).
(2) Prepare 10.0% solution of levulose, glucose, maltose, mannitol or sucrose.
(3) Sterilize (2) by heating at 100°C. either in a water bath or in a steamer for 2 minutes on three successive days.
(4) To 90 parts (1) add 10 parts (3). This makes the sugar concentration 1.0%.
(5) Pour (4) into sterile petri dishes in thin layers, and solidify.

Roth reported that all diphtheria bacilli fermented glucose and levulose. Some pseudo diphtheria bacilli fermented glucose and levulose.

(f) Abel (1912).
(1) Dissolve 1.0% peptone, 0.5% NaCl and 1.0% glucose in slightly alkaline meat infusion.
(2) Add 3 or 4 parts serum to 1 part (1).
(3) Mix well and tube.
(4) Solidify at a temperature between 90 and 95°C.

(g) Drigalski and Bierast (1913).
(1) Mix 600 cc. beef serum, 174 cc. 1% dextrose bouillon, 26 cc. bile.
(2) Pour into petri dishes in amounts of about 16 cc.
(3) Solidify at 90–95°C.
(4) Sterilize fractionally on 3 successive days as with Loeffler serum.

(h) Eastwood (1916) cultivated meningococci on the following medium:
(1) Medium is composed of 3 parts ox-blood serum, one part bouillon with 1.0% of glucose, galactose, malatose and sucrose respectively, and 1 in 10,000 of neutral red is added as an indicator. Details of preparation not given.

(i) Shimer (1916).
Add 1.4, 1.5 or 1.6 cc. of a 1.0% potassium tellurate solution to Loeffler's serum.

(j) Roddy (1917).
(1) Preparation of glucose bouillon not given.
(2) Mix 1 part of (1) with 3 parts of calf's or lamb's blood serum.
(3) Tube.
(4) Place in the serum sterilizer.
(5) Heat at 57°C. for 1 hour each day for 6 days and then at 70°C. 1 hour each day for 2 days or heat in

the Arnold steam sterilizer at 80°C. 1 hour each day for 3 days.

(k) Tanner (1919).
- (1) Collect beef blood at the abattoir in clean containers and place in the ice box until clot is formed.
- (2) Siphon or decant off the clear serum.
- (3) Add one part glucose broth (preparation not given) to three parts serum.
- (4) Tube.
- (5) Coagulate (method not given).
- (6) Sterilize in the autoclave at about 112°C. or in the Arnold using the intermittent method if desired.

(l) Harvey (1921–22).

Harvey added 2.0% or 3.0% chloroform to the serum as a preservative.

(m) Park, Williams & Krumwiede (1924).
- (1) Obtain beef or sheep blood from the slaughter house, and place in a sterile tall cylindrical vessel.
- (2) If the coagulum adheres to the sides of the vessel, loosen with a sterile glass rod.
- (3) Allow to stand for 24 hours on ice.
- (4) Pipette or siphon off the serum.
- (5) If bloody, place on ice and allow the corpuscle to settle out.
- (6) Mix 3 parts beef or sheep serum and 1 part nutrient broth (neutral to litmus at a pH = 6.8 to 7.0) to which 1.0% dextrose has been added.
- (7) Tube.
- (8) Slant the tubes in an apparatus where the temperature can be slowly raised to between 80 and 90°C. Do not heat above 95°C. until the medium is coagulated.
- (9) Sterilize in the Arnold for 20 minutes on each of 3 successive days.

(n) Park, Williams and Krumwiede (1924).
- (1) Add 0.5% glucose to heart infusion broth (see medium 794).
- (2) Mix 3 parts horse serum or plasma with 1 part (1).
- (3) Tube.
- (4) Slant.
- (5) Heat to 80 to 90°C. until coagulation is complete. Do not heat over 95°C.
- (6) Sterilize in the Arnold on each of 3 successive days for 20 minutes.

References: Loeffler (1887), Michel (1897 p. 261), Smith (1902, p. 104), Frost (1903 p. 138), Heineman (1905 p. 126), Rothe (1907 p. 620), Roux and Rochaix (1911 p. 247), Abel (1912 p. 25), Kolle Wasserman (1912 p. 406), Drigalski and Bierast (1913 p. 1237), Eastwood (1916 p. 408), Shimer (1916 p. 111), Roddy (1917 p. 43), Tanner (1919, p. 69), Ball (1919, p. 80), Besson (1920, p. 52), Harvey (1921–22 p. 78, 83), Dopter and Sacquépée (1921 p. 139), Giltner (1921 p. 367), Abbott (1921 p. 143), Pitfield (1922 p. 119), Stitt (1923 p. 41), Klimmer (1923 p. 221, 222), Park, Williams and Krumwiede (1924 p. 124).

2418. Rankin's Cyanide Glucose Serum

Constituents:

1. Bouillon....................	100.0 cc.
2. Serum, sheep................	300.0 cc.
3. Glucose...................	2.0 g.
4. Potassium-sulphocyanide....	4.0 g.
5. Neutral red (5.0% solution aqueous)....................	8.0 cc.

Preparation:
- (1) Method of preparation of bouillon not given.
- (2) Mix 3 parts good blood serum of the sheep and 1 part (1).
- (3) Add 3, 4 and 5 to (2).
- (4) Tube.

Sterilization: Coagulate and sterilize (exact method not given).

Use: Diagnosis of diphtheria. Author reported that *B. diphtheriae* always produced a pink colony which might be distinguished from cocci colonies.

Variants:

(a) Copelans gave the following method of preparation:
- (1) Exact composition and method of preparation of nutrient broth not given.
- (2) Obtain fresh sheep blood serum free from blood cells.
- (3) To the serum add 25.0% (1), 0.5% glucose, 1.0% of 1.0% watery neutral red solution and 1.0%

potassium sulphocyanide. ($CaCl_2$ may be added also if desired).

(4) Adjust to faint alkalinity.
(5) Distribute into test tubes.
(6) Sterilize and coagulate (method not specified).

Coplans reported that *B. diphtheriae* colonies after 18 hours were pink and a bluish pink tint diffused out into the medium. Medium originally yellow. 1.0% KCNS sufficed for this purpose. If 2.0% be added *B. Hofmanni* inhibited. If more than 2½% be added *B. diphtheria* and *Torulae* are inhibited.

(b) Coplans used 0.5 to 2.0% of Potassium-ferri-cyanide instead of 1.25 g. of potassium-sulpho-cyanide as in variant above. (0.5 to 2.0% KCNS may be added).
(c) Coplans used 1.0 g. potassium-sulphocyanide instead of 1.25 g. as in variant (a) above and added 0.5 to 2.0 g. boric acid. (The KCNS may be omitted.)
(d) Coplans added 1.0 g. Potassium ferrocyanide instead of 1.0% potassium sulpho-cyanide as in variant (a). (1.0 g. potassium sulpho-cyanide may also be added if desired).
(e) Harvey added 0.5% glucose, 1.0% potassium sulpho-cyanide, and 0.5% of a 2.0% neutral red solution to infusion broth, see variant (bb) 779. One part of this mixture was added to 3 parts sheep serum and coagulated.

References: Rankin (1911 p. 273), Coplans (1911 pp. 287, 282), Harvey (1921–22 p. 81).

2419. Ball's Glucose Bouillon Blood Albumin Medium

Constituents:

1. Glucose bouillon	85.0 cc.
2. Blood albumin (commercial)	15.0 g.

Preparation:

(1) Prepare glucose bouillon.
(2) Dissolve 15 parts commercial blood albumin in 85 parts (1).
(3) Tube.
(4) Inspissate in a slanting position.

Sterilization: Remove the tubes and sterilize by the fractional method in the steamer.

Use: General culture medium.

Reference: Ball (1919 p. 84).

2420. Crowe's Glucose Agar Blood Medium

Constituents:

1. Nutrient agar	250.0 cc.
2. Blood, defibrinated (bullock)	750.0 cc.
3. Glucose (1.0%)	10.0 g.

Preparation:

(1) Strain about a liter of defibrinated bullock blood thru muslin.
(2) Place 750.0 cc. of (1) in a water bath at 50°C.
(3) Melt 250.0 cc. of peptone (or trypsin) agar of the usual reaction.
(4) Mix (2) and (3) and add 1.0% glucose.
(5) Keep at 50°C. while plates or tubes are filled. At least 0.25 of an inch is required for each plate.
(6) Place in a steam sterilizer at 65 to 70°C. for an hour or two and then at 80–90°C. on each of 3 successive days.

Sterilization: See step (6) above.

Use: Differentiation of streptococci.

Reference: Crowe (1921 p. 486).

2421. Vardon's Bouillon Serum (Desiccated)

Constituents:

1. Bouillon	100.0 cc.
2. Horse serum	300.0 cc.

Preparation:

(1) Mix 1 part bouillon with 3 parts serum.
(2) Pour the mixture into shallow trays.
(3) Dry at a temperature not exceeding 50°C.
(4) Scrape the dried film off the trays and store for use.
(5) Dissolve the required amount of (4) in cold water. (Amount required to be calculated from the amount of serum and bouillon started with and amount of powder obtained).
(6) Distribute when dissolved in amounts of about 7.0 cc. into test tubes.
(7) Inspissate the medium in a sloping position at a temperature of 75°C. for 60 minutes on three successive days.
(8) Incubate tubes 24 hours and reject any which are not sterile.

Sterilization: Not specified.
Use: Cultivation of diphtheria bacilli.
Variants: The serum may be substituted by a solution of dessicated serum.
Reference: Vardon (1923–24 p. 431).

2422. Thalmann's Infusion Broth Serum Medium

Constituents:

1. Distilled water	2000.0 cc.
2. Beef	1000.0 cc.
3. Peptone (siccum)	20.0 g.
4. NaCl	10.0 g.
5. Serum (beef or hog)	

Preparation:

(1) Cut lean beef into small pieces in a meat cutting machine.
(2) Add a double weight of distilled water to (1).
(3) Boil for ¼ hour, stirring continually with a glass rod.
(4) Make up the loss of water and filter thru a filtering cloth.
(5) Add 1.0% peptone (siccum), and 0.5% NaCl.
(6) Boil.
(7) Make up the volume of water lost.
(8) Cool (in a closed container) and filter.
(9) Distribute in 300 to 500.0 cc. portions in clean flasks with patented sealers.
(10) Sterilize in streaming steam for one hour.
(11) Bring to boil on a concentrated salt solution bath and boil for 45 minutes shaking often.
(12) Take 30.0 cc. of (11), add a drop of alcoholic phenolphthalein solution and add N/1 sodium solution until a red coloration is formed.
(13) Estimate the amount of (11) and calculating from (12) add ⅔ to ¾ the amount of sodium solution required for neutralization.
(14) Place in warm water.
(15) Filter.
(16) Mix equal portions of serum (beef or hog, but hog serum preferred) and sterile (15).
(17) Tube.
(18) Place in a serum oven in a slanted position and heat on the first and second day for 2 hours at 70°C. and on the third day for one hour at 100°C.

Sterilization: Sterilization effected during preparation.
Use: Isolation of the gonococcus. The author reported that after 16 hours, gonococci colonies were round, dull typical colonies. The addition of sugar did not increase growth.
Variants: Klimmer mixed one part bouillon with three parts serum and coagulated at 65 to 80°C.
References: Thalmann (1900 p. 831), Klimmer (1923 p. 200).

2423. Ficker's Brain Infusion Serum Medium

Constituents:

1. Distilled water	500.0 cc.
2. Brain	500.0 g.
3. Serum	500.0 cc.
4. Glycerol	30.0 cc.

Preparation:

(1) Pass fresh brain (500.0 g.) thru a meat chopping machine.
(2) Add an equal weight of (500.0 g.) distilled water.
(3) Stir and heat slowly to a boil.
(4) Boil slowly for ¼ hour.
(5) Filter thru a filtering cloth until the filtrate assumes a pulpy character. Press the coagulated mass free from liquid.
(6) Mix equal parts of sterile (5) and serum.
(7) Add 3.0% glycerol.
(8) Tube.
(9) Solidify in a serum oven.

Sterilization: Steam the filtrate from (5) for two hours. Sterilization of other materials not specified.
Use: Cultivation of tubercle bacilli. Author reported that beef lungs, testicles, spleen, liver, kidney, udder, pancreas were treated in like manner but none gave as good results as brain. The lungs, spleen, liver and pancreas of man were used, but brain proved to be the best medium.
Variants: Klimmer prepared the medium as follows:

(1) Cut fresh brain into small pieces and add an equal weight of distilled water.
(2) Heat to boiling, stirring constantly.
(3) Filter thru a filtering cloth, and squeeze the meat free from juice.

(4) Sterilize for two hours (temperature not given).
(5) Add an equal volume of sterile solution of 3.0% glycerol in serum to (4) under aseptic conditions.
(6) Mix thoroly.
(7) Solidify quickly.

References: Ficker (1900 p. 593), Harvey (1921–22 p. 98), Klimmer (1923 p. 224).

2424. Ficker's Potato Juice Serum Medium

Constituents:

1. Potato juice	100.0 cc.
2. Serum (beef)	200.0 cc.
3. Glycerol (3.0%)	6.0 cc.

Preparation:
(1) Exact method of preparation of potato juice not given.
(2) Mix one part (1) with two parts beef serum.
(3) Add 3.0% glycerol.
(4) Reaction may be neutral or slightly alkaline or acid.
(5) Solidify in a serum oven.

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli. Author reported that best growth was obtained on neutral or slightly alkaline medium.

Reference: Ficker (1900 p. 509).

2425. Vannod's Nutrose Serum Medium

Constituents:

1. Distilled water	40.0 to 50.0 cc.
2. Serum (hog)	15.0 cc.
3. Glycerol	3.0 cc.
4. Nutrose	1.0 cc.

Preparation:
(1) Obtain hog blood from the abattoir.
(2) After 24 hours syphon the serum from the blood cells.
(3) Filter the serum thru a Berkefeld candle to free it from red corpuscles.
(4) Add 40, 45 or 50.0 cc. distilled water to 15.0 cc. of the filtered serum and mix thoroly.
(5) Add 3.0 cc. of glycerol and 1.0 cc. of nutrose to each flask of (4).
(6) Heat by a flame, and shake until boiling.

Sterilization: Sterilize three successive times in steam at 102°C.

Use: Cultivation of gonococci.

Reference: Vannod (1907 p. 13).

2426. Pergola's Egg Yolk Serum Medium

Constituents:

1. Ox serum	50.0 cc.
2. NaCl (0.8% soln.)	50.0 cc.
3. Potassium tellurite (1.0% soln.)	2.0 cc.
4. Egg yolk	1

Preparation: Details of preparation not given in the abstract.

Sterilization: Not given.

Use: Diagnosis of diphtheria.

Reference: Pergola (1918 p. 101 taken from 1919 p. 57).

2427. Greenspon's Glucose Citrate Serum Medium

Constituents:

1. Glucose veal infusion broth to	100.0 cc.
2. Serum, pig, sheep or human	75.0 cc.
3. Sodium citrate (50.0% soln.)	1.0 cc.

Preparation:
(1) Add 1.0 cc. of a 50.0% sodium citrate solution to 75.0 cc. of clear pig, sheep or human serum and sufficient glucose veal infusion broth to bring the volume to 100.0 cc.
(2) Adjust to pH 6.4 by the addition of 3.0% citric acid.
(3) Tube.
(4) Coagulate in the Koch inspissator.

Sterilization: Sterilize the fractional method on each of three successive days.

Use: Isolation and cultivation of diphtheria bacillus.

Variants: Beef extract broth may be used instead of veal infusion.

Reference: Greenspon (1922 p. 32).

2428. Duval's Egg Serum Medium

Constituents:

1. Distilled water	200.0 cc.
2. Human blood	100.0 cc.
3. Glycerol	10.0 cc.
4. Eggs	12

Preparation:
(1) Mix 200.0 cc. distilled water, 100.0 cc. of human blood serum and 10.0 cc. of glycerol.
(2) Carefully separate the whites of 12 eggs from the yolks and drop each separately and aseptically into a flask containing sterile (1).

(3) Shake thoroly.
(4) Tube carefully.
(5) Heat the tubes in a slanted position at a temperature not exceeding 70°C. until coagulation has taken place.
(6) Paraffin the cotton plugs to keep the medium moist.

Sterilization: Sterilize (1) by the intermittent method in the Arnold sterilizer.

Use: Isolation of tubercle bacilli.

Reference: Duval (1909 p. 404).

2429. Ebeling's Serum Tissue Plasma Medium

Constituents:

1. Distilled water	90.0 cc.
2. Plasma, chicken	10.0 cc.
3. Serum, chicken	
4. Embryonic tissue juice	
5. Acetic acid	1.0%

Preparation:

(1) Dilute 10.0 cc. of normal adult chicken plasma with 90.0 cc. of sterile distilled water. Shake thoroly in an Erlenmeyer flask, while adding 1.0 cc. of a 1.0% acetic acid solution drop by drop.
(2) Allow to precipitate partially in the cold for about an hour.
(3) Shake the contents of the flask and pour into centrifuge tubes 25.0 cc. in each tube.
(4) Centrifuge for 10 minutes and decant the supernatant fluid.
(5) Invert the tubes over a sterile piece of filter paper for complete drainage.
(6) Make up the precipitate contained in the tubes to 2.5 cc. with sterile distilled water.
(7) When (6) is thoroly mixed it has the appearance of rich milk.
(8) Mix $\frac{1}{4}$ part of (7) with $\frac{3}{4}$ chicken serum and one volume of embryonic tissue extract, by drawing them up and expelling them from a bulb pipette. Such a preparation has a pH value between 7.0 and 7.3 and coagulates in about 1 minute.

Sterilization: Method not given.

Use: Cultivation of connective tissue.

Reference: Ebeling (1921 p. 643).

2430. Wolbach and Schlesinger's Tissue Plasma Medium

Constituents:

1. Plasma, guinea pig.
2. Brain (testicle) guinea pig.

Preparation:

(1) Obtain guinea pig plasma by bleeding a guinea pig directly from the heart. The anasthetization of the guinea pig must be such as to have no reflex movements of the animal, but still have a vigorous heart action.
(2) As soon as collected (in a sterile paraffined tube) the blood is placed in an ice and salt mixture and chilled for a period of about 3 minutes.
(3) Centrifuge surrounded by an ice and salt mixture for a period of 3 to 5 minutes.
(4) Return to the salt and ice mixture (temperature just above the freezing point).
(5) Collect the supernatant plasma by means of chilled sterile paraffined pipette and transfer to another sterile chilled paraffined tube, and keep in this tube in the ice and salt bath at −5° to −7°C. If the plasma freezes, thaw out slowly. It may still be used.
(6) Obtain the tissue bran or testicles for cultivation with aseptic precautions, and keep immersed in sterile Ringer's solution until the plasma is prepared.
(7) Cut into pieces 0.5 to 1.0 cmm. in size under Ringer's solution.
(8) Transfer to sterile coverslips and cover immediately with a drop of sterile plasma (5).
(9) As soon as the plasma has clotted, the coverslips are inverted into hollow ground slides and sealed with sterile vaseline.

Sterilization: Method not given.

Use: Cultivation of *Dermacentroxenus rickettsi* (causing Rocky Mountain Spotted Fever) and *Rickettsia prowazeki* (causing typhus). To make transplants, remove the bits of tissue from the plasma, wash a few minutes in sterile Ringer's solution, transfer to fresh sterile coverslips and add fresh sterile plasma.

(Authors have kept tissue cultures from the testicles and brain of guinea pigs in active growth for four to six generations over a period of four to six weeks). Use guinea pig brain for growing typhus organism and guinea pig testicles for the cultivation of Rocky Mountain Spotted Fever microörganism. For injection into animals the tissue cultures are removed from the coverslips, placed in Ringer's solution and teased into pieces small enough to pass thru a number 18-gauge hypodermic needle.

Reference: Wolbach and Schlesinger (1923, p. 233).

SUBGROUP V-C

Solidifying Agent Egg or Egg Derivatives, etc.

Basal or Complete Media Solidified by Materials other than Blood or its Derivatives (Egg or Egg Derivatives, etc.) Initially Liquid but Becoming Permanently Solid

A_1. Solidified by whole egg.	
B_1. Additional constituents, if any, of known chemical composition.	
Dal Pozzo's Lapwing Egg Medium	2431
Ball's Boiled Egg Medium	2432
Abel's Egg Medium	2433
Smith's Egg Medium	2434
Dorset's Egg Medium (Heinemann)	2435
Modified Dorset's Egg Medium (Brown and Smith)	2436
Dorset's Egg Medium (Abel)	2437
Soparkar's Glycerol Egg Medium	2438
Lubenau's Glycerol Egg Medium (Abel)	2439
Twort and Ingram's Basal Glycerol Egg Medium	2440
B_2. At least one additional constituent of unknown chemical composition present.	
Roddy's Glucose Bouillon Egg Medium	2441
Putnam and Gay's Milk Egg Medium	2442
Petroff's Gentian Violet Egg Medium	2443
Harvey's Sperm Egg Medium	2444
Harvey's Olive Oil Egg Medium	2445
Lubenau's Glycerol Bouillon Egg Medium (Park and Krumwiede)	2446
Corper's, et al., Gentian Violet Egg Medium	2447
Twort's Bacteria Infusion Egg Medium	2448
Harvey's Trypsinized Heart Egg Medium	2449
Rosenthal and Schulz's Meat Infusion Egg Medium (Zimmermann)	2450
A_2. Solidified by egg white.	
Thoinot and Masselin's Egg Albumin Medium	2451
Barthel's Egg Albumin Medium	2452
Dal Pozzo's Transparent Egg Albumin	2453
Rosenthal and Schulz's Alkaline Egg Albumin (Zimmermann)	2454
Barthel's Milk Egg Albumin Medium	2455
Brown and Orcutt's Veal Infusion Egg Albumin Medium	2456
A_3. Solidified by yolk of egg.	
Pergola's Tellurite Egg Yolk Medium	2457
Smith's Egg Yolk Medium	2458
McCoy and Chapin's Egg Yolk (Francis)	2459
Dorset's Egg Yolk Medium (Heinemann)	2460
Nastinkoff's Egg Yolk Medium (Rechtsamer)	2461
Dal Pozzo's Egg Yolk Medium	2462
Lubenau's Bouillon Egg Yolk Medium (Schoenburg)	2463
Phisalix's Potato Egg Yolk Medium	2464
A_4. Solidified by other materials.	
Steffen's Sputum Medium	2465
McCann's Ovarian Cyst Fluid Medium	2466

2431. Dal Pozzo's Lapwing Egg Medium

Constituents:

1. Water	1000.0 cc.
2. Egg (lapwing)	100.0 cc.

Preparation:

(1) Wash lapwing's eggs with a 1% sublimate solution. (One egg will make about 4 or 5 tubes of medium.)

(2) Open the egg under aseptic conditions and allow the white of the egg to flow into a sterile flask.

(3) Mix the egg albumin with its volume of sterile water.

(4) Distribute into sterile test tubes.

(5) Coagulate in the same manner as the coagulation of serum, (details of method not given) at a temperature of about 70°C.

Sterilization: Method of sterilization of water or containers not given.

Use: Cultivation of saprophytic and parasitic forms.

Variants: The author added a sterile solution of glycerol, dextrin, or any other carbohydrate to the medium.

Reference: Dal Pozzo (1888 p. 526).

2432. Ball's Boiled Egg Medium

Constituents:
1. Egg.

Preparation:
(1) Boil eggs.
(2) Remove the shell over a small portion.

Sterilization: Not specified.

Use: General culture medium. The coagulated albumin was stroked with the inoculum.

Variants: Various authors have described similar media, and prepared them as follows:
(a) Bezançon.
  (1) Boil an egg.
  (2) Remove the shell.
  (3) Cut into discs.
  (4) Place the discs in Petri dishes.
  (5) Sterilization not specified.
(b) Besson.
  (1) Boil an egg.
  (2) Remove the shell.
  (3) Cut the egg into thin discs and place in Petri dishes.
  (4) Heat at 115°C. to sterilize.
(c) Harvey.
  (1) Place whole eggs in 10 N sodium hydroxide for 10 days.
  (2) Remove the shells.
  (3) Cut the eggs into fine slices.
  (4) Wash for 2 hours in running water.
  (5) Steam 60 minutes.
  (6) Transfer each slice of egg by means of sterile forceps to a sterile Petri dish.
  (7) Sterilize 20 minutes at 100°C. 3 days.
(d) Klimmer.
  (1) Boil eggs.
  (2) Cut into strips or prisms.
  (3) Add several drops of sterile water to each piece.
  (4) Sterilize (method not given).

References: Ball (1919 p. 84), Bezançon (1920 p. 121), Besson (1920 p. 54), Harvey (1921–22 p. 85), Klimmer (1923 p. 223).

2433. Abel's Egg Medium

Constituents:
1. Egg.

Preparation:
(1) Carefully wash and brush the shell of an egg.
(2) Wash in a 5.0% sublimate solution and in sterile water.
(3) Dry with sterile wool.
(4) Make a small opening in the apex of the egg.
(5) After inoculation close the opening with sealing wax or sterile paper and collodion.

Use: General culture medium.

Variants:
(a) Roddy prepared the medium as follows:
  (1) Shake thoroly a fresh (not more than 24 hours old) egg, having a perfect shell with no crack.
  (2) Wash in warm sterile water, then with warm 1:500 bichloride solution and again with sterile water.
  (3) Wipe dry.
  (4) Inoculate by piercing the shell with a sterile needle and introducing the bacteria with a platinum loop.
  (5) Seal the opening with sterile sealing wax.
  (6) Coat the egg with paraffin to obtain anaerobic conditions.
(b) Bezançon gave the following method of preparation:
  (1) Sterilize the shell of an egg.
  (2) Make a small hole in the egg shell near the region of the air chamber.
  (3) Inoculate.
  (4) Seal the opening with wax.

References: Abel (1912 p. 26), Roddy (1917 p. 45), Bezançon (1920 p. 121).

2434. Smith's Egg Medium

Constituents:
1. Egg.

Preparation:

(1) Thoroly mix the white and yolk of an egg before breaking the shell by shaking the egg vigorously.
(2) Remove the contents of the egg under aseptic conditions.
(3) Distribute into sterile test tubes.
(4) Coagulate by heat.

Sterilization: Method of sterilization of test tubes not given.

Use: Cultivation of plant parasites.

Reference: Smith (1905 p. 49).

2435. Dorset's Egg Medium (Heinemann)

Constituents:

1. Egg.

Preparation:

(1) Break eggs in a flask.
(2) Break the yolk with a platinum wire.
(3) Shake the flasks gently. Do not cause to foam.
(4) Tube in 10.0 cc. quantities.
(5) Heat in a Koch inspissator for 2 successive days for 4 or 5 hours at 70°C.

Sterilization: Not specified.

Use: General culture medium.

Variants: The following authors prepared similar media as indicated.

(a) Abel.
    (1) Carefully wash and brush the shell of an egg.
    (2) Wash in a 5.0% sublimate solution and in sterile water.
    (3) Dry with sterile wool.
    (4) Make an opening in either pole of the egg and blow the contents into a sterile test tube, flask or plates.
    (5) Coagulate in the same manner as blood serum.
(b) Waksman studied the metabolism of actinomycetes on the following medium:
    (1) Sterilize egg on surface.
    (2) Transfer contents into sterile containers.
    (3) Mix with sterile spatula.
    (4) Tube, slant, coagulate.
    (5) Sterilize in Arnold at 75 to 90° for 3 consecutive days.
(c) Scales studied the variation in morphology of *B. coli* in a medium prepared as follows:
    (1) Strain fresh eggs 4 times thru four thicknesses of cheese cloth.
    (2) Pour into a small suction flask and place under a vacuum for 5 minutes with occasional shaking.
    (3) Distribute in test tubes (0.75 cc. in tube 4 inches by ½ inch) with a pipette.
    (4) Coagulate in slanting position in boiling water or in an inspissator.
    (5) After coagulated, cover egg with cold distilled sterile water.
    (6) Plug and autoclave at 16 pounds for 30 minutes.
    (7) Pour off water just before using.

References: Heinemann (1905 p. 130), Abel (1912 p. 27), Tanner (1919 p. 58), Waksman (1919 p. 307), Scales (1921 p. 595), Pitfield (1922 p. 120).

2436. Modified Dorset's Egg Medium (Brown and Smith)

Constituents:

1. Distilled water	25.0 cc.
2. Whole egg	100.0 cc.

Preparation:

(1) Sterilize the shell of eggs.
(2) Mix, in sterile container, the white and yolk thoroly after removing from the shell.
(3) Add sterilized distilled water equal in amount to 24.0% by volume.
(4) Tube, avoiding air bubbles.
(5) Slant and heat to 85°C. in the ordinary blood serum coagulator on 3 successive days.

Sterilization: Not specified.

Use: Isolation of tubercle bacilli from sputum by the use of antiformin.

Variants: The following investigators prepared the medium as follows:

(a) Park and Krumwiede.
    (1) Wash eggs thoroly in water and then in 5.0% phenol solution and allow to partially dry.
    (2) Dry the ends of the eggs gently in the flame and pierce with a burned sharp forcep. The hole at one end should be about ⅜ of an inch in diameter and the membrane broken. The other end which is to be blown into should be smaller and the membrane left unbroken.
    (3) Blow the contents of the egg into a sterile Erlenmeyer flask.

(4) To the egg is then added 10.0% of water by volume of the weight of the eggs.
(5) Mix the eggs and water by a twirling motion or by gently stirring with a sterile glass rod. Bubbling is to be sedulously avoided.
(6) Strain (5) thru a cheese cloth by gravity and tube.
(7) Inspissate the tubes at 70°C. for 2 to 2½ hours in a moist chamber.
(8) If needed, moisture is provided by the addition of two or three drops of water to each tube.
(9) Incubate one week before using to insure sterility of medium.
(10) After inoculation, seal the tube either with cork alone or preferably dip the cotton stopper also in paraffin. The authors added glycerol bouillon to the slants until the egg was about one-third covered.

(b) Schoenburg.
(1) Remove the yolk and whites from eggs, under aseptic conditions. Mix well.
(2) Add 25.0% by volume of sterile distilled water to (1).
(3) Tube.
(4) Solidify at 85°C.
(5) Sterilize on 3 successive days for 2 to 3 hours at 85°C.

(c) Soparkar.
(1) Sterilize the surface of one dozen eggs by pouring boiling water over them.
(2) Break the shells with sterile forceps and pour contents into a sterile flask.
(3) Add 6.0 cc. (sterile ?) water for each egg (72.0 cc.) and thoroly mix by continued shaking.
(4) Strain material thru sterile muslin and distribute in tubes.
(5) Heat in inspissator on the first day up to about 90°C. for 3 hours until the medium becomes solid. Then at 72°C. for 3 hours on each of 2 succeeding days.

(d) Roddy.
(1) Wash clean fresh eggs with perfect shells in sterile water, then with 5.0% phenol, and finally with sterile water.
(2) Break with a sterile knife and empty the eggs into a sterile flask under aseptic conditions.
(3) Add 10.0% (by weight) of sterile distilled water.
(4) Mix (avoid air bubbles).
(5) Filter thru gauze.
(6) Tube.
(7) Slant.
(8) Sterilize in the steam sterlizer at 70°C. for 2.5 hours.

(e) Stitt.
(1) Break whole eggs into a sterile flask and mix thoroly.
(2) Add 25.0 cc. of water for every 4 eggs.
(3) Mix well.
(4) Strain thru sterile cheese cloth and tube in 10.0 cc. quantities.
(5) Slant in an inspissator and keep at 73°C. for 4 or 5 hours on 2 successive days.
(6) On the third day apply a temperature of 76°C.
(7) Add 3 or 4 drops of water to each tube before inoculating.
(8) Paraffin the plugs after inoculation.

(f) Klimmer.
(1) Thoroly wash eggs in water and then in 5.0% phenol solution.
(2) Dry both ends in the flame and open with a sterile knife.
(3) Force the contents of the eggs into sterile Erlenmeyer flasks.
(4) Add water to 10.0% of the weight of the eggs.
(5) Mix thoroly, avoiding air bubbles.
(6) Filter thru filtering cloth.
(7) Tube.
(8) Heat for 2 to 2.5 hours at 70°C. in an atmosphere saturated with water to coagulate.

References: Brown and Smith (1910 p. 517), Park and Krumwiede (1910 p. 213–215), Schoenburg (1911–12 p. 487), Soparkar (1916–17 p. 34), Roddy (1917 p. 45), Stitt (1923 p. 42), Klimmer (1923 p. 223), Park, Williams and Krumwiede (1924 p. 120).

2437. Dorset's Egg Medium (Abel)

Constituents:

1. Physiological salt solution...	100.0 cc.
2. Egg........................	300.0 cc.

Preparation:

(1) Wash absolutely fresh eggs in dilute formalin.
(2) Allow the shells to dry and break the eggs into a sterile dish.
(3) Thoroly mix the yolk and albuminous portion by gentle stirring.
(4) Strain the mixture thru sterile gauze.
(5) Add one part sterile physiological salt solution (0.85% NaCl) to 3 parts (4).
(6) Tube.
(7) Coagulate in 3 to 5 minutes by heating in the steamer.

Sterilization: Sterilize the tubes by heating at 105° for 20 minutes on each of 2 successive days. Screw all of the valves of the autoclave down before starting to heat.

Use: Cultivation of tubercle bacilli.

Variants:

(a) Abel stated that sterile glycerol bouillon might be added to the medium to prevent drying.
(b) Harvey prepared the medium as follows:
  (1) Clean thoroly the eggs to be used.
  (2) Wash with 5.0% carbolic acid.
  (3) Allow to dry partially.
  (4) Heat the ends of the eggs gently in the flame to dry them.
  (5) Pierce, with sterile precautions, both ends.
  (6) Blow the whole contents of the eggs into a sterile, wide-mouthed, glass stoppered bottle containing glass beads.

  NOTE: The eggs may be carefully broken and the contents allowed to fall into the bottle.

  (7) Add* 1 volume 0.85 sterile salt solution for each 3 volumes egg contents.

  *NOTE: If desired 3.75 cc. N/1 sodium hydroxide per 100.0 cc. egg mixture may be added and also 1.0% glucose at this stage.

  (8) Shake well to mix.
  (9) Strain thru cloth.

  NOTE: At this stage, if desired, some drops of alcohol basic fuchsin to give a slight pink color to the medium may be added.

  (10) Distribute into test tubes.
  (11) Coagulate.
  (12) Fill in each test tube 0.85 sterile salt solution to cover the medium.
  (13) Sterilize.

  NOTE: It is very usual to dispense with the addition of salt solution and subsequent sterilization. In that case sterility should be tested before use by incubation for 48 hours.

  (14) Pour off the salt solution at the time of use.

References: Abel (1912 p. 97), Harvey (1921–22 p. 84).

2438. Soparkar's Glycerol Egg Medium

Constituents:

1. Water with 5.0% glycerol.	72.0 cc.
2. Eggs......................	1.0 dozen

Preparation:

(1) Sterilize surface of one dozen eggs by pouring boiling water over them.
(2) Break the shell with sterile forceps and pour contents into a sterile flask.
(3) Add 6.0 cc. of (sterile ?) water containing 5.0% glycerol for each egg (72.0 cc.) and thoroly mix by continued shaking.
(4) Strain material thru sterile muslin and distribute in tubes.
(5) Heat in inspissator on the first day up to about 90°C. for 3 hours till the medium becomes solid. Then at 72°C. for 3 hours on each of two succeeding days.

Sterilization: Method not given.

Use: Cultivation of tubercle bacilli.

Reference: Soparkar (1916–17 p. 34).

2439. Lubenau's Glycerol Egg Medium (Abel)

Constituents:

1. Physiological salt solution...	100.0 cc.
2. Egg........................	300.0 cc.
3. Glycerol (6.0%)............	6.0 g.

Preparation:

(1) Wash absolutely fresh eggs in undiluted formalin.
(2) Allow the shells to dry and break the eggs into a sterile dish.

(3) Thoroly mix the yolk and albuminous portion by gently stirring.
(4) Strain the mixture thru sterile gauze.
(5) Add 1 part sterile physiological salt solution (0.85%) NaCl containing 6.0% glycerol to 3 parts (4).
(6) Tube.
(7) Coagulate in 3 to 5 minutes by heating in the steamer.

Sterilization: Methods not given.

Use: Cultivation of tubercle bacilli.

Variants: The author added sterile glycerol bouillon to prevent drying.

References: Abel (1912 p. 97), Harvey (1921–22 p. 85), Cunningham (1924 p. 165).

2440. Twort and Ingram's Basal Glycerol Egg Medium

Constituents:

1. Physiological salt solution....	30.0 cc.
2. Glycerol.....................	4.0 cc.
3. Eggs (fresh)..................	66.0 cc.

Preparation:

(1) Add 4.0 cc. of glycerol to 30.0 cc. of physiological salt solution.
(2) Add one of the added nutrients (amount not given) to (1).
(3) Steam for one-half hour.
(4) Add 66.0 cc. of whole freshly laid eggs. Mix well.
(5) Distribute into sterile tubes.
(6) Plug with cotton wool and sterilize at 61°C. for one hour on 3 successive days. Incubate the tubes over night between the second and third heating.
(7) Immediately after the third heating slope the tubes and inspissate at about 85°C.
(8) Inoculate and incubate for 4 months at 39°C.

Sterilization: See step (6) above.

Use: Cultivation of Johnes bacilli. Author reported that media prepared from vegetable extracts were not so good as media prepared from acid fast bacilli or their extracts. Growth was sometimes obtained with the extracts listed. Many others were tried with negative results.

Added nutrients: Alcoholic extracts of currant grapes, figs, oats, linseed, acid fast bacilli, and *Contharellus aurantiacus* (a fungus) were prepared using a Soxhlet extractor. The materials were extracted for 3 hours, filtered and the extract evaporated to dryness. One of these extracts was added to the basic medium.

Reference: Twort and Ingram (1914 p. 278).

2441. Roddy's Glucose Bouillon Egg Medium

Constituents:

1. Bouillon..................	1000.0 cc.
2. Glucose 1.0%..............	10.0 g.
3. Egg	

Preparation:

(1) Prepare a 1.0% glucose bouillon.
(2) Add 10.0 to 15.0 cc. of (1) to the white and yolk of one egg.
(3) Make a smooth mixture in a mortar.
(4) Tube.
(5) Inspissate.

Sterilization: Sterilize as for ordinary serum slants. (Method not given).

Use: General culture medium.

Variants:

(a) Roddy added 1.0 cc. of glycerol bouillon to each tube before final sterilization in the autoclave when used to cultivate tubercle bacilli. The cotton plugs were paraffined.
(b) Stitt cultivated anaerobes and prepared the medium as follows:
  (1) Preparation of 1.0% glucose bouillon not given.
  (2) Flame a mortar wet with alcohol.
  (3) Crack a clean egg with a sterile knife and allow the contents to drop into the sterile mortar.
  (4) Add from 10.0 to 15.0 cc. of sterile (1) to (3).
  (5) Add 5 drops of a 1.0% neutral red solution for each egg added to (4).
  (6) Mix into a smooth paste.
  (7) Inspissate for 2 hours in the rice cooker. If the mortar and knife are not sterilized it is necessary to sterilize after inspissation.
(c) Harvey cultivated anaerobes and isolated *B. diphtheriae* on the following medium.
  (1) Add 1.0% glucose to infusion broth (See variant (bb) medium 779).
  (2) Make a suspension of 12.0 cc. of (1) and the contents of one egg.
  (3) Place the suspension in test tubes.
  (4) Coagulate.

(5) Sterilize 20 minutes at 100°C. 3 days, 24 hours after completion of coagulation.
(6) Test sterility before use.

(d) Harvey also added 1.0 cc. glycerol to each test tube before final sterilization to convert into a medium suitable for cultivation of *B. tuberculosis* The addition of a few drops of 1.0% neutral red gives a color to the medium which serves to show up colonies, and serves as an indicator of the production of acid.

(e) Stitt cultivated tubercle bacilli on the following medium:
(1) Prepare a 1.0% glucose bouillon.
(2) Flame a mortar wet with alcohol.
(3) Crack a clean egg with a sterile knife and allow the contents to drop into the sterile mortar.
(4) Add from 10.0 to 15.0 cc. of sterile (1) to (3).
(5) Mix into a smooth paste.
(6) Inspissate for 2 hours in the rice cooker. If the mortar and knife are not sterilized it is necessary to sterilize after inspissation.
(7) One cc. of glycerol bouillon may be added to each tube before final sterilization.
(8) Plug the tubes with a rubber cap, or preferably, heat the end of the tube, withdraw the plug quickly and dip the part of the plug that enters the tube into hot melted paraffin. Then quickly reintroduce the plug.

References: Roddy (1917 p. 43), Harvey (1921–22 p. 85), Stitt (1923 p. 42).

2442. Putnam and Gay's Milk Egg Medium

Constituents:

1. Litmus milk	200.0 cc.
2. Egg	100.0 cc.
3. Glycerol (5.0%)	15.0 cc.
4. Peptone (0.8%)	2.4 g.
5. Beef extract (0.4%)	1.2 g.
6. Glucose (1.2%)	3.6 g.

Preparation:
(1) Mix one part egg with two parts litmus milk.
(2) Add 5.0% glycerol, 0.8% peptone, 0.4% beef extract and 1.2% glucose.
(3) Adjust to 1.14% acid—indicator not specified.
(4) Inspissate and sterilize in the Arnold.

Sterilization: See step (4) above.

Use: To determine growth of influenza bacilli in mixed cultures. Author reported that medium does not support the growth of the influenza bacillus in mixed culture.

Reference: Putnam and Gay (1920–21 p 4).

2443. Petroff's Gentian Violet Egg Medium

Constituents:

1. 15.0% glycerol in water.	500.0 cc.
2. Beef (or veal)	500.0 g.
3. Whole egg	1000.0 cc.
4. Gentian violet	1 to 10,000.

Preparation:
(1) Infuse 500.0 g. beef or veal in 500.0 cc. of a 15.0% solution of glycerol in water for 24 hours.
(2) Squeeze in a sterile meat press and collect in a sterile beaker.
(3) Sterilize the shells of eggs by immersing for 10 minutes in 70.0% alcohol or by pouring hot water upon them.
(4) Break the eggs into a sterile beaker and mix.
(5) Filter thru a sterile gauze.
(6) Add two parts by volume of (5) to one of (2).
(7) Add sufficient 1.0% alcoholic gentian violet to make a dilution of 1 to 10,000.
(8) Tube in 3.0 cc. lots in sterile test tubes and inspissate for 3 successive days, 85°C. the first day. On the second and third days for not more than one hour at 75°C.

Sterilization: Method not given.

Use: Isolation and cultivation of tubercle bacilli. For the cultivation of bovine tubercle bacilli, the meat was infused in plain water, glycerol not being added. Waksman cultivated actinomyces on a medium similarly prepared.

Variants: The following investigators prepared the medium as follows:

(a) Keitly prepared the medium like above. To sterilize, however, he placed the tubes in the inspissator. Bring the temperature to 95°C. as quickly as possible, avoiding bubbles, and keep at 95° for one hour. Repeat this inspissating at 95°C. for one hour on 2 successive days. Seal tubes by dipping the cotton plug into a hot mixture of 7 parts paraffin and 1 part bees wax and 1 part petrolatum.

(b) Roddy.
- (1) Infuse 500.0 g. veal in 500.0 cc. of a 15.0% solution of glycerol in water for 24 hours.
- (2) Strain off the juice.
- (3) Sterilize the shells of eggs with 20.0% phenol.
- (4) Break the eggs into sterile beakers and mix well. Avoid air bubbles.
- (5) Filter thru gauze.
- (6) Add one part of (2) to one part of (5) by volume.
- (7) Add sufficient 1.0% alcoholic gentian violet to make a 1:10,000 dilution.
- (8) Tube.
- (9) Sterilize in the steam sterilizer or inspissate for 3 successive days at 80° for one hour each day.

(c) Waksman cultivated actinomyces on the following medium:
- (1) Prepare 500.0 cc. of meat juice containing 15.0% glycerol.
- (2) Mix 1000.0 cc. beaten eggs, (1) and 1:10,000 gentian violet.
- (3) Tube.
- (4) Slant.
- (5) Coagulate.
- (6) Sterilize in the Arnold at 75 to 90° for 3 consecutive days.

(d) Limousin.
- (1) To 250.0 g. of fresh sterile crushed veal and 212.0 g. of distilled water and 37.5 g. sterile glycerol.
- (2) Place in the ice box over night and filter thru sterile gauze, under aseptic conditions.
- (3) Sterilize the shell of 16 to 20 eggs by immersing in alcohol for 15 minutes.
- (4) Open the eggs under aseptic conditions and remove the yolk and white of the eggs.
- (5) Thoroly mix the yolk and white under aseptic conditions.
- (6) Mix 400.0 cc. of (2) and 400.0 cc. of (5) under aseptic conditions.
- (7) Dissolve 0.5 g. gentian violet in 50.0 cc. of 95% alcohol.
- (8) Add 1.0 cc. of (7) for each 100.0 cc. of (6) to (6) under aseptic conditions.
- (9) Distribute in sterile tubes.
- (10) Heat on 3 successive days as follows:
  85° for 30 minutes on the 1st day.
  75° for 30 minutes on the 2nd day
  75° for 30 minutes on the 3rd day.

Limousin stated that all apparatus, etc., coming in contact with this medium must be sterile, and preparation carried on under aseptic conditions.

(e) Despeignes.
- (1) Grind 250.0 g. of veal in a sterile grinder.
- (2) Mix 212.0 cc. distilled water and 37.5 g. of sterile glycerol with (1).
- (3) Allow to stand over night and filter thru sterile muslin.
- (4) Sterilize by heating on 8 successive days for one hour at 55 to 57°C.
- (5) Place the necessary number of eggs in alcohol at 70°C. for 15 minutes.
- (6) Break the egg and mix the contents in a sterile container with a sterile glass rod.
- (7) Filter (6) thru sterile muslin.
- (8) Mix 200.0 cc. of (4) with 400.0 cc. of (7) under aseptic conditions.
- (9) Add 6.0 cc. of a 1.0% solution of gentian violet in 95% alcohol to (8).
- (10) Mix well.
- (11) Distribute in sterile tubes.
- (12) Slant the tubes and heat at 85° for 30 minutes.
- (13) Heat at 75°C. for 30 minutes on the two following days.
- (14) Incubate 3 days at 37°C. to test sterility.

(f) Stitt.
- (1) Treat 500.0 g. of chopped up meat with 500.0 cc. of 15.0% glycerol solution.
- (2) Keep in the ice chest for 24 hours.
- (3) Filter thru gauze.
- (4) Sterilize the shells of 2 eggs by immersing in 70.0% alcohol for 10

minutes or by dipping in boiling water for 5 seconds or so.
(5) Mix the whites and yolks of these eggs in a sterile mortar.
(6) Add 1.0% alcoholic solution of a 1:100 gentian violet solution to the filtrate of (3).
(7) Mix equal parts (6) and (5).
(8) Tube in 3.0 to 4.0 cc. quantities and slant.
(9) Inspissate at 85° until the medium has solidified.
(10) Heat the slants at 75°C. for one hour on the second and third days.

(g) Klimmer.
(1) Soak 250.0 g. of finely chopped lean veal in 212.0 cc. of water and 37.5 g. glycerol in the ice box over night.
(2) Filter 16 to 20 fresh thoroly mixed eggs thru gauze.
(3) Add 200.0 cc. of the juice from (1) to 400.0 cc. of (2).
(4) To each 100.0 cc. of (3) add 1.0 cc. of a 1.0% gentian violet solution.
(5) Distribute in tubes.
(6) Heat in the serum inspissator on each of 3 successive days for 30 minutes at 85°, 75° and 75°C.

(h) Park, Williams and Krumwiede.
(1) Extract 500.0 g. of veal or beef in 500.0 cc. of 15.0% glycerol in water for 24 hours.
(2) Collect the fluid by means of a press.
(3) Sterilize by filtering.
(4) Add 1.0 cc. of a 1.0% alcoholic solution of gentian violet to each 100.0 cc. of (3).
(5) Thoroly cleanse egg with water.
(6) Wash with 2.5% carbolic acid solution.
(7) Allow partially to dry.
(8) Dry the ends in the flame, and pierce with a small flamed sharp forcep. The hole should be ⅜ of an inch in diameter and the membrane broken.
(9) Make a smaller hole in the other end and do not break the membrane. This hole is to blow into.
(10) Blow the contents of the eggs into sterile Erlenmeyer flasks. Blow from the cheeks.
(11) Mix 1 part (4) to 2 parts (10).
(12) Tube in sterile tubes.
(13) Inspissate on 3 days for 45 minutes at 80 to 85°C.

References: Petroff (1915 p. 39), Roddy (1917 p. 46), Waksman (1919 p. 307), Giltner (1921 p. 368), Limousin (1921 p. 559), Harvey (1921–22 p. 85), Despeignes (1922 p. 931), Stitt (1923 p. 47), Klimmer (1923 p. 224), Park, Williams and Krumwiede (1924 p. 127).

2444. Harvey's Sperm Egg Medium

Constituents:
1. Physiological Salt Solution.
2. Glycerol.
3. Sperm.
4. Egg.

Preparation:
(1) Add 5.0% sperm oil to beaten and filtered egg medium.
(2) Add glycerol 0.85 sterile salt solution to give 5.0% of glycerol.
(3) Shake the mixture in a bowl of hot water with a Bunsen burner underneath, to melt the wax.
(4) Distribute into test tubes and keep in a bowl of hot water to prevent the wax separating out.
(5) Shake to mix.
(6) Place low down in a steamer and steam 4 minutes to coagulate the egg.

Sterilization: Sterilize by placing high up in the steamer for 1 hour on 3 successive days.

Use: Cultivation of tubercle bacilli.

Reference: Harvey (1921–22 p. 87).

2445. Harvey's Olive Oil Egg Medium

Prepare in the same manner as Harvey's Sperm Egg Medium, see medium 2444, but use olive oil instead of sperm.

2446. Lubenau's Glycerol Bouillon Egg Medium (Park and Krumwiede)

Constituents:

1. Whole eggs	10
2. 5.0% glycerol bouillon	250.0 cc.

Preparation:
(1) Sterilize the outside of 10 eggs by washing with water and then 5.0% phenol.

(2) Dry the ends of the eggs gently in a flame.

(3) Pierce the ends with a burned sharp forcep. The end which is to be blown into should be smaller and the membrane to be left unbroken.

(4) Blow the contents of the egg into a flask containing 250.0 cc. of 5.0% glycerol bouillon, neutral or moderately alkaline to litmus.

(5) Mix by a twirling motion or by gently stirring with a sterile glass rod. Bubbling is to be sedulously avoided.

(6) Strain thru a cheese cloth by gravity and tube.

(7) Inspissate the tubes at 70°C. for 2 to 2¼ hours, in a moist chamber.

(8) Incubate one week to insure sterility of media.

Sterilization: Sterilization given under preparation.

Use: Cultivation of tubercle bacilli. Park and Krumwiede sealed the tube with cork alone or preferably dipped the cotton stopper in paraffin and then stoppered with cork. Waksman studied the metabolism of actinomycetes on a similarly prepared medium.

Variants:

(a) Waksman used the following medium:

  (1) Mix 400.0 to 600.0 cc. egg and 1000.0 cc. bouillon containing 5.0% glycerol.

  (2) Tube, slant and coagulate.

  (3) Sterilize in Arnold at 75° to 90° for 3 consecutive days.

(b) Giltner prepared the medium as follows:

  (1) Wash thoroly 4 eggs with water and wash in 5.0% phenol.

  (2) Allow the eggs to get partially dry.

  (3) Dry the ends of the eggs in a flame.

  (4) Break the ends with a pair of sterile forceps, breaking the membrane in one end and leaving it intact in the other.

  (5) Expell the contents of the eggs by blowing in the end with the unbroken membrane into sterile Erlenmeyer flasks.

  (6) Break the yolks with a sterile wire platinum and thoroly mix the whites and yolks.

  (7) Add 25.0 cc. of distilled water.

  (8) Strain thru a sterile cloth.

  (9) Distribute into sterile test tubes.

  (10) Allow the tubes to stand in water for 10 to 15 minutes at 45°C. to drive out the air.

  (11) Slant in an inspissator and expose to 70° to 75°C. for 4 or 5 hours on 2 successive days.

  (12) Gradually raise the temperature above this on the third day and finish sterilization by a single exposure at 100°C. in an Arnold sterilizer.

  (13) Before inoculation add 2 or 3 drops of sterile broth to each tube.

  (14) If human tubercle bacilli are to be cultivated, add 5.0% glycerol.

References: Park and Krumwiede (1910 p. 215), Waksman (1919 p. 307), Giltner (1921 p. 368), Klimmer (1923 p. 223), Park, Williams and Krumwiede (1924 p. 120).

2447. Corper's, et al., Gentian Violet Egg Medium

Constituents:

1. Water	1000.0 cc.
2. Glycerol	200.0 g.
3. NaCl	20.0 g.
4. Beef extract (Liebig's)	12.0 g.
5. Peptone (Witte or Difco)	40.0 g.
6. Eggs	3000.0 cc.
7. Gentian violet	1:10,000

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Adjust reaction to neutral to litmus.

(3) Mix whites and yolks of eggs.

(4) Mix ¼ volume of (2) and 3 volumes of (3).

(5) Add gentian violet (Grübler's) 1:10,000.

Sterilization: Inspissate or autoclave to sterilize, 10–15 pounds for 30 to 60 minutes.

Use: Cultivation of tubercle bacilli from sputum.

Reference: Corper, Fiala and Kallen (1918 p. 270).

2448. Twort's Bacteria Infusion Egg Medium

Constituents:

1. Infusion solution.

2. Egg.
3. Glycerol.
4. *Bacillus phlei.*

Preparation:

(1) Grind up 1.0 g. of dried *Bacillus phlei* in 12.0 cc. of glycerol and 22.0 cc. of infusion broth (saline or peptone beef broth may be used).

(2) Autoclave (1).

(3) Add (2) when cool to 66.0 cc. of mixed yolk and white of fresh hen eggs.

(4) Tube.

Sterilization: Sterilize on each of three successive days at 61°C. and inspissate in slopes.

Use: Cultivation of Johns bacillus, human lepra bacillus and a spirochaete. *Bacillus phlei* was cultivated on 4.0% glycerol infusion broth.

Reference: Twort (1921 p. 798).

2449. Harvey's Trypsinized Heart Egg Medium

Constituents:

1. Water	1000.0 cc.
2. Heart, horse	500.0 g.
3. Egg	3000.0 cc.

Preparation:

(1) Beat up the contents of several fresh eggs.

(2) Mix 3 parts (1) with 1 part trypsinized heart prepared as given under the variants of medium 1117.

(3) Filter thru muslin.

(4) Tube.

(5) Coagulate.

Sterilization: Method not given.

Use: General culture medium.

Reference: Harvey (1921–22 p. 85).

2450. Rosenthal and Schulz's Meat Infusion Egg Medium (Zimmermann)

Constituents:

1. Egg white.
2. Meat infusion.

Preparation:

(1) Prepare meat infusion.

(2) Filter egg albumin thru a double layer of muslin by hand under the application of pressure.

(3) Place the clear filtrate in a measuring cylinder fitted with a ground glass stopper.

(4) Add to 5.0 cc. of albumin, 2.2 cc. of a sterile 1.0% solution of NaOH or KOH and 2.8 cc. of sterile meat infusion.

(5) Allow to stand one hour, and shake back and forth gently to mix.

(6) Distribute into sterile test tubes or other sterile culture containers.

(7) Place the containers in hot (95°–98°) water for a short time to coagulate the albumin.

Sterilization: Method not given.

Use: General culture medium.

Variants: The author reported that salts as NaCl, Na_2CO_3, K_2CO_3, Na_2SO_4, Na_2HPO_4, etc., may be added if desired.

Reference: Rosenthal and Schulz (1888 p. 307), Zimmermann (1888 p. 314).

2451. Thoinot and Masselin's Egg Albumin Medium

Constituents:

1. Egg albumin.

Preparation:

(1) Remove the albumin of an egg under aseptic conditions by means of a pipette.

(2) Distribute directly into sterile test tubes.

(3) Coagulate as serum or at 100°C.

Sterilization: Method not given.

Use: Cultivation of *B. pyocyaneus.* Other investigators cultivated a large number of organisms on similar media.

Variants: The following investigators prepared the medium as indicated:

(a) Smith cultivated plant parasites on the following medium:

(1) Thoroly flame the end of the egg from which the egg albumin is to be poured.

(2) Distribute the albumin in sterile test tubes.

(3) Coagulate in the same manner as blood serum.

(b) Duval isolated *B. leprae* on the following medium. He reported that the colonies at first were greyish white but after several days they assumed a distinct orange yellow tint. Transfers may be made from this medium to slants or other plates.

(1) Pour egg albumin (human blood serum), (or nutrient agar with or without 1.0% tryptophane may be

used) into sterile Petri dishes and inspissated for 3 hours at 70°C.

(2) An excised leprous nodule is then cut into thin slices, 2 to 4 millimeters in breadth and 0.5 to 1.0 mm. in thickness and distributed over the surface of the coagulated albumin or serum.

(3) Bathe the medium then seeded, with a 1.0% sterile trypsin solution, taking care not to submerge the pieces of leprous tissue. Add sufficient fluid to moisten thoroly the surface of the medium.

(4) Incubate in a moist chamber at 37°C. for a week to ten days. Remove the plates from time to time and add more trypsin as evaporation necessitates.

(c) Besson.

(1) Sterilize the shell of an egg by washing in a sublimate solution.

(2) Dry with sterile filter paper.

(3) Heat the end of the egg in a flame until the shell is black.

(4) Make a small hole in this end using a flamed knife point.

(5) Aspirate the white of the egg using a sterile pipette.

(6) Distribute in sterile tubes.

(7) Coagulate by heating at 70°C. as for the coagulation of serum.

References: Thoinot and Masselin (1902 p. 53), Smith (1905 p. 48), Duval (1911 p. 369), Abel (1912 p. 27), Besson (1920 p. 54), Harvey (1921–22 p. 86).

2452. Barthels Egg Albumin Medium

Constituents:

1. Water.
2. Egg albumin.

Preparation:

(1) Cut egg albumin in small cubes. (Passini-Achalm's method, details not given.)

(2) Place in water in Gruber's tubes.

Sterilization: Sterilize for 15 minutes at 125°C.

Use: Cultivation of anaerobic bacteria from milk. Author inoculated the tubes with milk that had been fermented under anaerobic conditions, and removed the air from the tubes.

Reference: Barthel (1910 p. 6).

2453. Dal Pozzo's Transparent Egg Albumin

Constituents:

1. Water.
2. Egg albumin.

Preparation:

(1) Clean the eggs thoroly and open.

(2) Catch the thin albuminous liquid flowing out first in a sterile container.

(3) Add $\frac{1}{4}$ portion of water.

(4) Distribute into test tubes.

(5) Slant.

Sterilization: Sterilize by the discontinuous method in the steamer.

Use: Cultivation of saprophytic and parasitic organisms.

Variants: The author reported that glycerol, dextrin, etc., might be added as needed.

Reference: Dal Pozzo (1888 p. 151).

2454. Rosenthal and Schulz's Alkaline Egg Albumin (Zimmermann)

Constituents:

1. Water.
2. Egg albumin.
3. KOH (1.0% solution).

Preparation:

(1) Filter egg albumin thru a double layer of muslin by hand under the application of pressure.

(2) Place the clear filtrate in a measuring cylinder fitted with a ground glass stopper.

(3) Add to 5.0 cc. of albumin, 3.0 cc. of a 1.0% solution of KOH and 2.0 cc. of water.

(4) Allow to stand an hour, shaking only back and forth, to mix the alkali, egg and water.

(5) Distribute into sterile test tubes or other sterile culture containers.

(6) Place the containers in hot water (95°–98°) for a short time to coagulate the albumin.

Sterilization: Method not specified.

Use: General culture medium.

Variants:

(a) Rosenthal and Schulz used NaOH instead of KOH.

(b) Salts such as NaCl, Na_2CO_3, K_2CO_3, Na_2SO_4, Na_2HPO_4, etc., may be added.

Reference: Rosenthal and Shulz (1888 p. 307), Zimmermann (1888 p. 314).

2455. Barthel's Milk Egg Albumin Medium

Constituents:

1. Water	1000.0 cc.
2. Glucose (1.0%)	10.0 g.
3. Egg albumin	
4. Milk	

Preparation:

(1) Add 0.1 cc. milk to 5.0 cc. of a 1.0% glucose solution.

(2) Add (1) to Gruber's tubes containing sterile egg white.

Sterilization: Sterilize (2) at 125 to 130°C. (Method not given).

Use: Cultivation of anaerobic bacteria from milk.

Reference: Barthel (1910 p. 6).

2456. Brown and Orcutt's Veal Infusion Egg Albumin Medium

Constituents:

1. Veal infusion broth	1000.0 cc.
2. Egg albumin	3000.0 cc.

Preparation:

(1) Prepare standard veal infusion broth.

(2) Mix 3 parts egg albumin with 1 part (1).

(3) Tube.

(4) Slant and coagulate in the inspissator.

Sterilization: Not specified.

Use: Cultivation of *Bacillus pyogenes*.

Reference: Brown and Orcutt (1920 p. 224).

2457. Pergola's Tellurite Egg Yolk Medium

Constituents:

1. Egg yolk	75.0 g.
2. Glycerol	12.5 g.
3. NaCl (0.8% soln.)	12.5 cc.
4. Potassium tellurite	0.1 g.

Preparation: Method not given in abstract.

Sterilization: Method not given in abstract.

Use: Cultivation of diphtheria bacilli.

Reference: Pergola (1925 p. 537) taken from (1925 p. 420).

2458. Smith's Egg Yolk Medium

Constituents:

1. Egg yolk.

Preparation:

(1) Remove the yolks from eggs under aseptic conditions.

(2) Distribute into sterile test tubes.

(3) Coagulate in a slanting position at 80°C.

Sterilization: Not specified.

Use: Cultivation of plant parasites.

Variants: The whole egg may be boiled hard, the yolk removed and cut into pieces with a sterile knife and distributed into sterile Petri dishes.

Reference: Smith (1905 p. 49).

2459. McCoy and Chapin's Egg Yolk (Francis)

Constituents:

1. Saline solution (40.0%)	40.0 cc.
2. Egg yolk (60.0%)	60.0 cc.

Preparation:

(1) Thoroly wash fresh hen eggs in soap, water and alcohol. Remove all the alcohol from the egg by igniting the alcohol.

(2) Cut the egg in two using a sterile sharp knife and separate the white by decanting them from one half the shell to the other, thus allowing the whites to drain from the yolks.

(3) Collect the yolks in a sterile beaker.

(4) Add 40.0% sterile saline solution to 60.0% egg yolk.

(5) Mix thoroly.

(6) Tube using a sterile funnel.

(7) Place tubes in metal racks constructed so as to allow one-half inch space between the tubes for circulation.

(8) Heat the racked tubes in a slanting position for the first half hour at 70°C. and for the second half hour at 72°C.

(9) Substitute sterile paraffined corks for the cotton plugs.

(10) Incubate the tubes in an upright position for 3 or 4 days to test sterility.

Sterilization: Method of sterilization of saline solution not given.

Use: Cultivation of *Bacterium tularense*.

Reference: Francis (1922 p. 103).

2460. Dorset's Egg Yolk Medium (Heinemann)

Constituents:

1. Water.
2. Egg yolk.

Preparation:

(1) Add 5.0 to 10.0 g. of sterile water to the yolks of 3 or 4 eggs.

(2) Shake the flasks gently.

(3) Tube in 10.0 cc. quantities.
(4) Heat in a Koch inspissator on 2 successive days for 4 or 5 hours at 70°C.

Sterilization: Not specified.

Use: General culture medium.

Reference: Heinemann (1905 p 130).

2461. Nastinkoff's Egg Yolk Medium (Reaktsamer)

Constituents:

1. Distilled water	600.0 cc.
2. Egg Yolk	300.0 cc.
3. NaOH (1.0%)	100.0 cc.

Preparation:

(1) To 300.0 cc. of egg yolk add 600.0 cc of sterile distilled water and 100.0 cc. 1.0% sterile NaOH solution.
(2) Distribute into test tubes and heat on a water bath to 75–85°C.
(3) Heat once more to 85°C. which is sufficient to sterilize the coagulated transparent medium.

Sterilization: Method of sterilization not given.

Use: Cultivation of diphtheria, cholera, influenza and other pathogenic forms.

Reference: Nastinkoff (1893 #33 and 34), Reaktsamer (1895 p. 492).

2462. Dal Pozzo's Egg Yolk Medium

Constituents:

1. Water.
2. Egg yolk.

Preparation:

(1) Collect egg yolk from thoroly cleaned eggs.
(2) Add (1) to ¼ volume sterile water (may contain glycerol).
(3) Filter.
(4) Distribute into sterile test tubes.
(5) Slant.

Sterilization: Method of sterilization of water not given. Sterilize the slants in the steamer using the discontinuous method.

Use: Cultivation of saprophytic and parasitic organisms.

Variants: Glycerol, dextrin, etc., may be added as desired.

Reference: Dal Pozzo (1887 p. 523).

2463. Lubenau's Bouillon Egg Yolk Medium (Schoenburg)

Constituents:

1. Bouillon.
2. Glycerol.
3. Egg yolk.

Preparation:

(1) Wash eggs in hot soap solution.
(2) Wash in alcohol and place in Petri dishes.
(3) Burn off the alcohol.
(4) Make a small hole in the end of the egg by means of a sterile glass knife.
(5) Allow the egg white to run out.
(6) Place the egg yolk in a sterile flask containing small pieces of glass.
(7) Shake well.
(8) Add an equal volume of the ordinary sterile filtered bouillon containing 3.0% glycerol and neutralized with soda.
(9) Mix thoroly.
(10) Tube.
(11) Solidify at 85 to 90°C. in a serum apparatus by heating on 3 days for 2 or 3 hours.

Sterilization: Method of sterilization of (8) not given.

Use: Cultivation of tubercle bacilli. Other investigators, using glucose instead of glycerol, cultivated diphtheria bacilli.

Variants: Kolle and Wasserman prepared the medium as follows:

(1) Thoroly wash eggs with hot soapy water, place in alcohol for several minutes and then burn the alcohol from the eggs after they have been removed.
(2) Make a small opening in the egg shell and allow the white to run out.
(3) Remove the yolks and place in a sterile flask.
(4) Mix 100.0 cc. of neutral to litmus meat infusion solution with the yolks of 5 or 6 eggs.
(5) Add 1.0% glucose (to cultivate diphtheria bacilli) or 3.0% glycerol (to cultivate tubercle bacilli) to (4).
(6) Solidify by heating at 90° on each of 3 successive days for 2 or 3 hours.

References: Schoenburg (1911–12 p. 487), Kolle and Wassermann (1912 p. 414), Besson (1920 p. 54), Harvey (1921–22 p. 86).

2464. Phisalix's Potato Egg Yolk Medium

Constituents:

1. Potato purée.
2. Glycerol.
3. Egg Yolk.

Preparation:

(1) Prepare a potato purée containing a small amount of glycerol (method not given).

(2) Add egg yolk to (1) until a soft paste is obtained.

(3) Spread (2) in tubes or plates.

Sterilization: Sterilize in the autoclave. A solid mass forms.

Use: Cultivation of tubercle bacilli. Author reported that it may be necessary to moisten the surface of the egg yolk medium by the addition of sterile water or glycerol bouillon.

Reference: Phisalix (1903 p. 604).

2465. Steffen's Sputum Medium

Constituents:

1. Sputum.

Preparation:

(1) Remove all visible impurities from human sputa.

(2) Heat (temperature not given) and remove the dirt floating on top. (The addition of water not specified.)

(3) Place in test tubes and plates and solidify (Method not given).

Sterilization: Not specified.

Use: Cultivation of pneumococci, streptococci, staphylococci and diphtheria bacilli.

Reference: Steffen (1895 p. 464).

2466. McCann's Ovarian Cyst Fluid Medium

Constituents:

1. Ovarian cyst fluid.

Preparation:

(1) Collect the fluid from ovarian cyst during an abdominal operation by means of a sterilized trocar under aseptic conditions. Reject the first portion of the fluid, and collect the remainder in tall cylindrical sterile glass jars.

(2) The jars should be fitted with accurately fitting glass stoppers. They should be cleaned with a 1.0% mercuric chloride solution followed by absolute alcohol. The stopper should be smeared with vaseline containing corrosive sublimate.

(3) Allow the fluid to stand in the ice box for 24 hours.

(4) Pipette off the supernatant fluid by means of a sterile pipette and transfer to sterilized test tubes provided with a sterile cotton stopper.

(5) Slant and heat at 66 to 68°C. for 4 hours.

(6) Incubate at 36° to test sterility.

(7) After inoculation place the tubes into a glass vessel half filled with distilled water, and provided with a well fitted glass plate.

Sterilization: Method not given.

Use: Cultivation of gonococci.

Reference: McCann (1896 p. 1491).

GROUP VI. INITIALLY LIQUID MEDIA BECOMING PERMANENTLY SOLID. SOLIDIFYING AGENT INORGANIC

A_1. Additional materials, if any, inorganic.

B_1. Basal media; employed with the addition of other nutrients.

- Beijerinck and van Delden's Basal Silicate Jelly 2467
- Smith's Basal Silicate Jelly 2468
- Stevens and Temple's Basal Silicate Jelly 2469
- Doryland's Basal Silicate Jelly 2470

B_2. Complete media.

C_1. Nitrogen supplied as ammonium salts.

- Sleskin's Ammonium Sulphate Silicate Jelly 2471
- Omelianski's Ammonium Sulphate Silicate Jelly 2472
- Beijerinck's Ammonium Sodium Phosphate Silicate Jelly 2473

C_2. Nitrogen supplied as nitrates or nitrites.

A_2. At least one constituent organic.

B_1. All additional materials of known chemical composition.

- Doryland's Basal Glucose Silicate Jelly 2474
- Sullivan's Basal Glycerol Silicate Jelly 2475
- Fermi's Solution Silicate Jelly (Smith) 2476
- Doryland's Basal Acetic Acid Silicate Jelly 2477
- Waksman and Carey's Cellulose Silica Jelly 2478

B_2. At least one additional constituent of unknown chemical composition.

C_1. Solidified with silicic acid.

- Kühne's Beef Extract Silicate Jelly (Kerry) 2479

C_2. Solidified by materials other than silicic acid (gypsum).

- Omelianski's Gypsum Block 2480
- Omelianski's Nitrite Gypsum Block (Smith) 2481
- Omelianski's Mannitol Gypsum Block (Percival) 2482
- Makrinoff's Leaf Infusion Gypsum Block 2483
- Makrinoff's Soil Infusion Gypsum Block 2484
- Omelianski and Makrinoff's Soil Infusion Gypsum Block (Löhnis). 2485

2467. Beijerinck and van Delden's Basal Silicate Jelly

Constituents:

1. Water glass.
2. HCl.

Preparation:

(1) Titrate a water glass solution, diluted with a known amount of water, with normal acid. Dilute the water glass so that there will be contraction when coagulation takes place. There must be complete neutralization for coagulation is hastened by an alkaline reaction.

(2) For example, add 5.0 cc. of commercial water glass to a clear beaker. Dilute with 25.0 cc. of water.

(3) To another beaker add 10.0 cc. of N acid (determined for this case).

(4) Mix the acid with the diluted water glass.

(5) Pour in plates.

(6) Free the solidified plate of NaCl by placing in a stream of tap water.

(7) Wash off with boiled water and pour over the nutrient salt solution.

(8) Gently heat the bottom of the plate until all the excess water is evaporated. The plate shows a dry glistening upper surface.

Sterilization: Flame off the surface whereby a partial but sufficient sterilization is obtained.

Use: Isolation of *B. oligocarbophilus*. The authors reported that the medium would also support the growth of nitrifying organisms. If chalk, $MgCO_3$ or NH_4MgPO_4 be mixed with the medium a snow white plate will be obtained. Such a plate will support the growth of the lower algae and earth diatomes.

Added nutrients: The authors added any desired nutrient salt solution.

Reference: Beijerinck and van Delden (1903 p. 38).

2468. Smith's Basal Silicate Jelly

Constituents:

1. Sodium silicate.
2. HCl.

Preparation:

(1) Add drop by drop 100.0 cc. of sodium silicate (sp. gr. 1.09) to 100.0 cc. of HCl (sp. gr. 1.10° Beaume) stirring the mixture constantly with a glass rod.

(2) Place in a collodion sac and dialyze until all traces of salt have been removed in running water.

(3) Add to this, sterile concentrated synthetic culture medium of any desired composition.

(4) Distribute in test tubes, Petri dishes or flasks.

Sterilization: Sterilize by heating for 3 hours in the blood serum oven at 90°C. on each of 3 successive days, or by heating in the autoclave at 110°C. for 15 minutes.

Use: Synthetic solid substrata.

Added nutrients: The author added any desired sterile concentrated synthetic culture solution.

Reference: Smith (1905 p. 37).

2469. Stevens and Temple's Basal Silicate Jelly

Constituents:

1. Water.
2. Sodium silicate.
3. HCl.

Preparation:

(1) Ascertain the amount of silicic anhydride in a sample of sodium silicate, by decomposing the silicate with concentrated HCl, precipitating the silicic acid, evaporating to dryness, washing until the wash water shows no chloride, then heat to redness and weigh the silicic anhydride.

(2) Dilute the silicate so that the solution contains 4.0 to 5.0% silicic anhydride.

(3) Prepare hydrochloric acid of such a strength so that 1.0 cc. neutralizes 1 cc. of the sodium silicate solution, using methyl orange as an indicator.

(4) To 104.0 cc. of this acid, add slowly and with constant stirring 100.0 cc. of the sodium silicate solution (the excess acid being used to prevent coagulation during sterilization).

(5) Tube.

(6) The jelly should be perfectly clear.

(7) To cause the sterile jelly to solidify add 1.0 cc. of a sterile concentrated solution of such salts as may be desired but in every case containing enough sodium carbonate to a little more than to neutralize the excess of acid present.

Sterilization: Sterilize (5) at 120° for 15 minutes in the autoclave.

Use: Solid synthetic medium. In preparing plates, inoculate before adding salts. This allows mixing before coagulating. If slants are desired slant immediately after adding the salts.

Added nutrients: The authors added a sterile concentrated solution of any desired carbohydrate.

Reference: Stevens and Temple (1908 p. 86).

2470. Doryland's Basal Silicate Jelly

Constituents:

1. Water		
2. HCl	1000.0	cc.
3. $MgSO_4$	0.5	g.
4. $CaCO_3$ or CaO	0.01	g.
5. $(Fe)_2(SO_4)_3$	0.01	g.
6. $MnSO_4$	0.01	g.
7. K_2SiO_3		
8. Na_2SiO_3		

Preparation:

(1) Prepare a mixture of K_2SiO_3 and Na_2SiO_3 dissolved in water so that the concentration be 34.2732 g. per liter.

(2) Dissolve 3, 4, 5 and 6 in HCl.

(3) Standardize (2) against the silicate solution so that 1.0 cc. of acid neutralizes 1.0 cc. of silicate jelly.

(4) Draw (1) into a plugged burette and allow to stand for several hours to sterilize.

(5) Draw the standardized acids into a plugged burette and allow to stand several hours to sterilize.

(6) Add 5.0 cc. of (5) to a sterile petri dish and then 5.0 cc. of (4) rotating rapidly to mix thoroly.

(7) Add any desired nutrient salts.

(8) Allow to stand. A gel forms.

Sterilization: See steps (4) and (5) above.

Use: Synthetic culture medium.

Added nutrients: The author added a concentrated sterile solution of any desired salts.

Variants: The author used HCl, H_2SO_4, and H_3PO_4 alone or in mixtures instead of HCl.

Reference: Doryland (1916 p. 144).

2471. Sleskin's Ammonium Sulphate Silicate Jelly

Constituents:

1. Water	1000.0 cc.
2. $(NH_4)_2SO_4$	4.0 g.
3. $MgSO_4$	0.5 g.
4. Potassium phosphate	1.0 g.
5. $CaCl_2$	trace
6. Na_2CO_3	6.0 to 9.0 g.
7. Water glass	
8. HCl	

Preparation:

(1) Pour 1 part diluted HCl (HCl with specific gravity of 1.17 one part with one part water) into 3 parts diluted water glass (specific gravity 1.08).

(2) Dialyze in running water until free from chlorine.

(3) Store sterile in a water free place until ready for use.

(4) Evaporate (3) in a platinum dish under a flame to a specific gravity of 1.02 (acid content 3.4%) or better until the fluid has become quite a thick liquid, about ½ the volume and needle like crystals can be seen swimming in different depths of the liquid. This is best carried out by measuring about ⅔ or ½ of the volume of the liquid making a mark on the wall of the container and then evaporate by boiling to this mark.

(5) Dissolve $(NH_4)_2SO_4$ and $MgSO_4$ in the least amount of water possible.

(6) Dissolve potassium phosphate and Na_2CO_3 in least amount of water possible.

(7) Prepare a very dilute solution of $CaCl_2$.

(8) Store sterile (5), (6) and (7) until ready for use.

(9) These salts may be mixed with various amounts of silicic acid. 1.15% to 1.45% salts give a fairly good nutrient jelly. If 2 to 3.0% of mineral salt be added solidification will take place much more rapidly. Instead of dissolving the salts in water and then adding the salt solution to the silicic acid, add the corresponding amount of water as the amount of mineral salts you desire in the medium to (4).

(10) Pour the sterilized (5) and (6) into (9) and mix well and finally a few drops of sterile weak $CaCl_2$ solution until the mixture becomes rather vicous.

(11) The liquid then is poured into sterile Petri dishes (it may be inoculated in liquid state) and allowed to stand at room temperature to solidify (requires several hours).

Sterilization: Method not specified.

Use: Cultivation of nitrifying organisms.

Variants:

(a) Burri and Stutzer prepared a similar medium as follows:

(1) Mix equal volumes of waterglass (sp. gr. 1.05) and HCl (sp. wt. 1.10).

(2) Dialyze in running tap water for 24 hours.

(3) Dialyze in distilled water for 4 days, changing the water three times a day.

(4) Evaporate 600.0 cc. of (3) to 150.0 to 200.0 cc. by boiling over a free flame.

(5) Transfer to sterile flask that is marked at 100.0 cc. Plug with cotton or close with asbestos and continue the evaporation until the 100.0 cc. volume mark is reached.

(6) Dissolve 10.0 g. KH_2PO_4, 5.0 g. $MgSO_4$, 10.0 g. NaCl and a trace of $CaCl_2$ in 1000.0 cc. water.

(7) Prepare a 10.0% $(NH_4)_2SO_4$ solution.

(8) Prepare a soda solution (strength not given).

(9) Sterilize (6), (7), (8), and (9), separately. (Method not given.)

(10) Add 10.0 cc. sterile (6) to sterile (5), and on the other side of the flask add sterile ammonia sul-

phate solution so that the ammonium sulphate is present in 1.0%. Then add the sterile soda solution in 2 to 4.0% strength.

(11) Mix well, may be inoculated if desired, and pour into sterile Petri dishes.

(12) Place over night in the incubator to solidify.

(b) Fremlin prepared a medium as follows:

(1) Add an equal amount of HCl, solution, sp. gr. 1.1 to a pure silicate of soda of a strength between 3.3 to 4.0%. (Add the acid to the silicate.)

(2) Dialyze 4 days in running tap water.

(3) Continue to dialyze all the chlorides from the mixture by dialyzing in distilled water.

(4) Pour the dialyzed mixture into a narrow necked flask and evaporate to about ½ its volume.

(5) Dissolve 0.4 g. $(NH_4)_2SO_4$, 0.05 g. $MgSO_4$, 0.1 g. Potassium phosphate, trace of $CaCl_2$ and 0.0 to 0.9 g. Na_2CO_3 in 100.0 cc. distilled water.

(7) Add to each dish about ½ the quantity of (5) as there is silicate solution.

(8) Evaporate the whole slowly over hot water until silicate coagulates.

(9) Sterilization not specified.

(c) Smith gave the following method of preparation:

(1) Dissolve 4.0 g. $(NH_4)_2SO_4$, 0.5 g. $MgSO_4$, 1.0 g. potassium phosphate, 6.0 to 9.0 g. Na_2CO_3 and a trace of $CaCl_2$ in the least possible amount of water.

(2) Add (1) to the dialyzed silicate jelly.

(d) Johnson prepared the medium as follows:

(1) Dissolve 0.4 g. $(NH_4)_2SO_4$, 0.5 g. $MgSO_4$, 0.1 g. K_2HPO_4, trace of $CaCl_2$ and 0.75 g. Na_2CO_3 in 100.0 cc. water.

(2) Sterilize (method not given).

(3) Pour a solution of commercial "waterglass" in dilute HCl.

(4) Dialize in a sausage parchment tube to get rid of the HCl.

(5) Sterilize the pure silicic acid solution (method not given).

(6) Evaporate (5) until a jelly is formed on mixing with (2). (Amount of (2) not given.)

(e) Abbott gave the following method of preparation:

(1) Prepare a 3.0 to 4.0% solution of silicic acid in distilled water (method not given.)

(2) Dissolve 0.4 g. $(NH_4)_2SO_4$, 0.05 g. $MgSO_4$, trace of $CaCl_2$ in 50.0 cc. of distilled water.

(3) Sterilize (2) method not given.

(4) Dissolve 0.1 g. potassium phosphate and 0.6 to 0.9 g. Na_2CO_3 in 50.0 cc. distilled water.

(5) Sterilize (4) method not given.

(6) Mix (3) and (5) when cool.

(7) Add (6) to (1), little by little, until the proper degree of consistency is obtained. This is best accomplished in a culture dish. If one desires to have isolated colonies, it is necessary to add the inoculum to the silicic acid before the addition of the salts.

(f) Winogradsky (Harvey) prepared the medium in the following manner:

(1) Prepare No. 1 solution: Ammonium sulphate 0.4; magnesium sulphate 0.05; calcium chloride 0.01; distilled water 50.

(2) Prepare No. 2 solution: Potassium phosphate 0.1; sodium carbonate 0.6; distilled water 50.

(3) Prepare No. 3 solution: Silicic acid 3.4; distilled water 100.

(4) Mix No. 1 and No. 2 solutions in equal quantities.

Note: The sterile nutrient mixture of Nos. 1 and 2 solutions may first be inoculated and then added with sterile precautions to sterile silicic acid (No. 3 solution) in a Petri dish. Rotate to mix and solidify.

(5) Add by degrees this mixture to No. 3 solution with constant stirring until solidification occurs.

(6) Distribute the solidified medium in plates.

(7) Sterilize 30 minutes at 100°C. on each of 3 successive days.

(g) Heinemann gave the following method of preparation:

(1) Mix 100.0 cc. of HCl (sp. gr. 1.10 Beaume's scale at 60°F.) with 100.0 cc. of sodium silicate (sp. gr. 1.09° Beaume's scale at 60°F.).

(2) Place (1) in colloidal sacs and dialyze in running water for 12 hours.

(3) Dissolve 0.4 g. $(NH_4)_2SO_4$, 0.05 g. $MgSO_4$, 0.1 g. KH_2PO_4, 0.9 g. Na_2CO_3 and 0.01 g. $CaCl_2$ in the smallest amount distilled water possible.

(4) Heat both (3) and (2) to boiling and cool rapidly without stirring.

(5) Mix the two solutions.

(6) Tube or pour into Petri dishes. (Slant the tubes.)

(7) Sterilize in the autoclave at 110°C.

References: Sleskin (1891 p. 210), Burri and Stutzer (1895 p. 723), Fremlin (1903 p. 271), Smith (1905 p. 198), Johnson (1912 p. 218), Abbott (1921 p. 604), Harvey (1921–22 p. 106), Heinemann (1922 p. 38).

2472. Omelianski's Ammonium Sulphate Silicate Jelly

Constituents:

1. Water	1000.0 cc.
2. HCl	100.0 cc.
3. Waterglass	100.0 cc.
4. Potassium phosphate	1.0 g.
5. $MgSO_4$	0.5 g.
6. $(NH_4)_2SO_4$	3.0 g.
7. $FeSO_4$	
8. NaCl	
9. $MgCO_3$	

Preparation:

(1) Mix equal volumes of waterglass (sp. gr. 1.05–1.06) and HCl (sp. gr. 1.10).

(2) Dialyze using parchment until all traces of chlorine have been removed. There are 2 parts SiO_3 in a 100 parts of this solution. The specific gravity at 16°C. measured with a pyknometic is 1.0121. This solution may be sterilized at 115–120°C.

(3) Dissolve 4, 5 and 6 in 1000.0 cc. distilled water.

(4) Prepare a 2.0% $FeSO_4$ solution.

(5) Prepare a saturated salt solution.

(6) Prepare milk of magnesia—that is a suspension of $MgCO_3$ in water.

(7) To 50.0 cc. of (2) add 2.5 cc. of (3) and 1.0 cc. of (4).

(8) Distribute into plates or tubes.

(9) Add all the way from a platinum loop to a small drop of (5) to each tube or plate and (6) may be added until the gel becomes milky. (Soda, about 0.1% may be used instead of $MgCO_3$.)

Sterilization: Not specified.

Use: Isolation of nitrifying organisms. The plates or tubes may be inoculated while the mixture is still a liquid, or after solidification takes place which requires about an hour.

Variants: Stevens and Temple (Percival) prepared a similar medium as follows:

(1) Take 3 to 4.0 cc. of a solution of sodium silicate (waterglass) and place in a porcelain dish.

(2) Add 5.0 to 10.0 cc. of a concentrated HCl to precipitate the silicic acid.

(3) Evaporate to dryness.

(4) Moisten again with HCl and evaporate to dryness again.

(5) Wash and transfer to a swedish filter paper, the weight of whose ash is known.

(6) Wash the precipitate until it gives no cloudiness when treated with silver nitrate solution.

(7) Burn the filter with the precipitate on it in a weighed porcelain crucible and heat to redness.

(8) Allow to cool and weigh the silicic anhydride (SiO_2).

(9) Calculate the amount of silicic anhydride in 1.0 cc. of the original solution.

(10) Dilute the amount of waterglass which is to be used for the preparation of the jelly until the solution contains 4 to 5.0% silicic anhydride.

(11) Prepare a hydrochloric acid of such strength that 1.0 cc. just neutralizes 1.0 cc. of (10) using methyl orange as an indicator.

(12) Take 104.0 cc. of (11) and add 100.0 cc. of (10).

(13) Distribute in tubes or flasks and sterilize at 120° for 15 minutes or at

least 100°C. for 20 minutes on 3 successive days.

(14) Dissolve 2.0 g. $(NH_4)_2SO_4$, 1.0 g. K_2HPO_4, 2.0 g. NaCl, 0.5 g. $MgSO_4$ 0.4 g. $FeSO_4$ and HCl in 1000.0 cc. distilled water and sterilize (method not given).

(15) Inoculate.

(16) To each 10.0 cc. of medium add 0.5 cc. of (14) under aseptic conditions.

(17) Slant or pour into plates.

References: Omelianski (1899 p. 541), Percival (1920 p. 146, 148), Harvey (1921–22 p. 106).

2473. Beijerinck's Ammonium Sodium Phosphate Silicate Jelly

Constituents:

1. Water.
2. Waterglass.
3. HCl.
4. $CaCO_3$.
5. NH_4NaHPO_4
6. KCl.

Preparation:

(1) Titrate commercial waterglass exactly with half normal HCl.

(2) Prepare a $CaCO_3$ solution.

(3) Determine the necessary amounts of 0.5 N HCl, Waterglass and $CaCO_3$ to mix together so that a suitable jelly is obtained.

(4) Measure these solutions into plates when ready for use and mix. Solidification will depend upon the ratio in which the reagents are mixed.

(5) Extract the soluble salts from the plates by pouring boiled distilled water over the plates.

(6) Add the desired amount of a solution containing NH_4NaHPO_4 and KCl with other desired salts.

Sterilization: Not specified.

Use: To study nitrite formation by *Amoeba nitrophila*.

Reference: Beijerinck (1896 p. 259).

2474. Doryland's Basal Glucose Silicate Jelly

Constituents:

1. Distilled water	500.0 cc.
2. K_2SiO_3 (C.P.)	24.0 g.
3. Na_2SiO_3 (C.P.)	8.4 g.
4. HCl dilute	
5. $MgSO_4$	0.5 g.
6. CaO	0.01 g.
7. $Fe_2(SO_4)_3$	0.01 g.
8. $MnSO_4$	0.01 g.
9. H_2SO_4	
10. H_3PO_4	
11. Glucose	

Preparation:

(1) Dissolve 8.4 g. Na_2SiO_3 and 24.0 g. K_2SiO_3 in 500.0 cc. distilled water.

(2) Dilute HCl to such a concentration so that 1.0 cc. of (1) does not quite neutralize 1.0 cc. of HCl. (Phenolphthalein as indicator.)

(3) Add the following salts to the HCl:

$MgSO_4$	0.5 g.
CaO	0.01 g.
$Fe_2(SO_4)_3$	0.01 g.
$MnSO_4$	0.01 g.

One of the added nutrients.

(4) Standardize (3) so that 1.0 cc. of (3) is equivalent to 1.0 cc. of (1). Methyl orange as indicator.

(5) Standardize solution of H_2SO_4 in same manner as HCl omitting the salts.

(6) Standardize H_3PO_4 in same way as HCl omitting salts and using phenolphthalein.

(7) Mix acids in following proportions:

HCl	153.5 cc.
H_2SO_4	77.0 cc.
H_3PO_4	116.0 cc.

(8) 1.0 cc. of (7) should neutralize 1.0 cc. of (1) using phenolphthalein as indicator.

(9) Draw (1) and (7) into plugged burettes and allow to stand several hours to sterilize.

(10) Draw 5.0 cc. of (7) and 5.0 cc. of (1) into a sterile Petri dish, add enough sterile aqueous glucose solution tc have 10.0 g. per liter of medium Rotate. Hardens in 5 minutes.

Sterilization: See step (9) above.

Use: Solid synthetic medium.

Added nutrients: The author added one of the following:

KNO_3
$K_6Fe_2(CN)_{12}$
$(NH_4)_2SO_4$.

Reference: Doryland (1916 p. 148).

2475. Sullivan's Basal Glycerol Silicate Jelly

Constituents:

1. Water	1000.0 cc.
2. Sodium silicate (4.0%)	40.0 g.
3. Glycerol (3.5%)	35.0 g.
4. H_3PO_4 (10.0%)	

Preparation:

(1) Add to the water about 4.0% sodium silicate.

(2) Add 3.5% glycerol which prevents the silicic acid from throwing out its water. Mix.

(3) Add drop by drop with frequent stirring enough of a 10.0% solution of H_3PO_4 sterile (2) to render slightly acid to phenolphthalein.

(4) Medium solidifies at room temperature in about 30 minutes. In the sterilizer the medium is solidified in from 5 to 10 minutes.

Sterilization: Method not given.

Use: To study pigment production.

Variants: A solution of any desired salts may be used instead of water.

Reference: Sullivan (1905–06 p. 118).

2476. Fermi's Solution Silicate Jelly (Smith)

Constituents:

1. Water	100.0 cc.
2. $MgSO_4$	0.2 g.
3. KH_2PO_4	1.0 g.
4. Ammonium phosphate	10.0 g.
5. Glycerol	45.0 g.
6. HCl	500.0 cc.
7. Sodium silicate	500.0 cc.

Preparation:

(1) Add drop by drop 500.0 cc. of sodium silicate (sp. gr. 1.09) to 500.0 cc. of HCl (sp. gr. 1.10° Beaume) stirring the mixture constantly with a glass rod.

(2) Place in a collodion sac and dialyze in running water until all traces of salt have been removed.

(3) Dissolve 2, 3 and 4 in 100.0 cc. of freshly boiled distilled water.

(4) Add 45.0 g. glycerol to (3).

(5) Boil (3) for a minute or 2 to drive off the absorbed air.

(6) Cool (5) to 50°C.

(7) Add 90.0 cc. of (4) to 500.0 cc. of (6). It is necessary to have (4) free from air. Either boil and cool or remove the air using an air pump. Mix thoroly by stirring with a clean glass rod.

(8) Pipette quickly into tubes.

Sterilization: Autoclave at 110°C. for 15 minutes.

Use: Synthetic medium for plant parasites

Reference: Smith (1905 p. 39).

2477. Doryland's Basal Acetic Acid Silicate Jelly

Same as medium 2474 but containing 153.5 cc. acetic acid instead of glucose. The acetic acid is added with the acids in step (7) and not at the end, as in the case of glucose.

2478. Waksman and Carey's Cellulose Silica Jelly

Constituents:

1. Distilled water	100.0 cc.
2. $(NH_4)_2HPO_4$	5.0 g.
3. $MgSO_4$	1.0 g.
4. $FeSO_4$	0.02 g.
5. KCl	1.0 g.
6. HCl	
7. Potassium silicate	
8. Cellulose (filter paper)	5.0 g.
9. $CaCO_3$	

Preparation:

(1) Prepare a normal solution of HCl and an equivalent solution of C.P. potassium silicate solution (about 10% silicate).

(2) Place 5.0 cc. portions of the acid in a series of tubes, and add varying quantities of silicate (4.5, 4.75, 5.0, 5.25, 5.75, 6.0, etc. cc.).

(3) Mix and pour into a series of dishes.

(4) Select the mixture that solidifies in from two to five minutes.

(5) Dilute the silicate solution with distilled water so that equal volumes of the silicate and HCl give a gel in the desired period of time.

(6) Pour from 100.0 cc. to 200.0 cc. of silicate solution into an equal volume of the acid solution and mix thoroly.

(7) Pour (6) into the lower halves of petri dishes in a layer 3 to 4 mm. deep.

(8) Allow the mixture to stand until a gel has formed.
(9) Place in deep flat vessels and dialyze in running tap water until free from chlorides. This requires about 24 hours.
(10) Transfer the dishes to a sterile vessel containing boiled distilled water. Replace this boiled distilled water several times.
(11) Dissolve 2, 3, 4 and 5 in 1.
(12) Grind filter paper.
(13) Add about 5.0 g. of (12) to (11).
(14) Pour about 2.0 cc. of (13) over each plate in such a manner so as to have the cellulose evenly distributed over the surface.
(15) Powder $CaCO_3$ on each plate.
(16) Place the uncovered plates at 65°C. until the excess of water has evaporated without allowing the plates to become dry.
(17) Cover the dishes with sterile tops.

Sterilization: Method of sterilization of (13) not given.

Use: To cultivate and isolate organisms capable of decomposing cellulose. Cellulose decomposing organisms after 48 to 72 hours produce yellow or orange colored growth around the soil particles, rapidly spreading over the plate. The yellow organism is *Spirochaeta cytophaga*.

Reference: Waksman and Carey (1926 p. 90).

2479. Kühne's Beef Extract Silicate Jelly (Kerry)

Constituents:

1. Water	
2. Sodium silicate	300.0 cc.
3. HCl (dilute)	100.0 cc.
4. Beef extract (Liebig's)	

Preparation:

(1) Mix 3 parts commercial, liquid sodium silicate (sp. gr. 1.08) with 1 part dilute HCl (1 part HCl with sp. gr. 1.17 to 1 part water).
(2) Dialyze in running water 4 days. Then may be dialyzed in distilled water if desired, to remove all NaCl.
(3) Concentrate the mixture over free flame in a platinum dish.
(4) Continue to heat until a thin membrane is formed. (Liquid has a sp. gr. of 1.02 and contains 3.4% pure acid.)
(5) Mix a piece of Liebig's extract (size of a bean) in 25.0 cc. of water.
(6) Add 0.5 or 1.0 cc. of (5) to 4.0 cc. of (4). (If NaCl be added, solidification will take place more rapidly.)

Sterilization: It is more satisfactory to sterilize the solutions separately and then mix. In an emergency, however, sterilize the mixture. (Method not given.)

Use: General culture medium.

Variants: Sodium chloride or glycerol may be added.

References: Kühne (1890 Heft 1), Kerry (1890 p. 410).

2480. Omelianski's Gypsum Block

Constituents:

1. Water	1000.0 cc.
2. Potassium phosphate	1.0 g.
3. $MgSO_4$	0.5 g.
4. $(NH_4)_2SO_4$	2.0 g.
5. NaCl	2.0 g.
6. $FeSO_4$	0.4 g.
7. Gypsum	
8. $MgCO_3$	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Add $MgCO_3$ in excess or the $MgCO_3$ may be added to the gypsum block.
(3) Prepare a completely homogeneous mixture of $MgCO_3$ and gypsum using 1.0% $MgCO_3$ usually.
(4) Add water to (3), stirring constantly until it reaches the consistency of acid cream.
(5) Pour on a large piece of plate glass and smooth out to an even thickness.
(6) When solidification starts, make circles (for Petri dishes) or narrow strips (for tubes). (Circles are made with a Petri dish.)
(7) When completely solidified, pass a knife between the circles or strips and glass to remove the solid gypsum. Equalize the uneven portions as much as possible.
(8) Place in Petri dishes (or tubes) smooth side up.
(9) Add (1) to the plate so that the level of solution is half way to the surface of the block.

(10) It may be necessary to add more sterile (1) to the block after sterilization.

Sterilization: Sterilize at 120°C. in the autoclave. Take care that the solution never gets on top of the block.

Use: Cultivation of nitrifying organisms.

Reference: Omelianski (1899 p. 653).

2481. Omelianski's Nitrite Gypsum Block (Smith)

Constituents:

1. Distilled water	1000.0 cc.
2. $MgCO_3$ (1.0%)	10.0 g.
3. Gypsum	1000.0 g.
4. $MgSO_4$	0.3 g.
5. $FeSO_4$	0.4 g.
6. NaCl	0.5 g.
7. K_2HPO_4	0.5 g.
8. Na_2CO_3	1.0 g.
9. $NaNO_2$ (Merck)	1.0 g.

Preparation:

(1) Dissolve 2, 3, 4, 5, 1.0 g. of fused Na_2CO_3 and 1.0 g. of Merck's $NaNO_2$ in 1.

(2) Uniformly mix 1.0% $MgCO_3$ with gypsum and water added to it until it becomes the consistency of sour cream.

(3) Pour upon a plate glass and spread out.

(4) When the mass assumes a doughy consistency, cut into circular discs for Petri dishes and into strips for test tubes.

(5) When the blocks have hardened, pry loose from the plate glass.

(6) Place in Petri dishes or in test tubes.

(7) Add sterile (1) to sterile (7) until the block is half immersed. Do not wet the inoculated surface.

Sterilization: Sterilize (6) in the autoclave. Method of sterilization of (1) not given.

Use: General synthetic culture medium.

Reference: Smith (1905 p. 199).

2482. Omelianski's Mannitol Gypsum Block (Percival)

Constituents:

1. Water	
2. Gypsum (plaster of Paris)	
3. K_2HPO_4	0.05 g.
4. NaCl	0.05 g.
5. Mannitol	2.0 g.

Preparation:

(1) Mix water and Gypsum (plaster Paris) to the consistency of cream (about 8 volumes of gypsum powder to 3.0 cc. water).

(2) Pour on a plate of glass in a layer of 1 inch thick.

(3) Before the mixture has set cut into narrow strips 3 inches long by 0.5 to 0.75 inches broad, or into circular pieces.

(4) Dissolve 0.5 g. K_2HPO_4, 0.5 g. NaCl, and 2.0 g. mannitol in 100.0 cc. water.

(5) Add 5.0 cc. of (4) to test tubes, and place a strip of gypsum in each tube or pour a few cc. of (4) into a Petri dish, and place a circular piece of (3) in the plate.

Sterilization: Not specified.

Use: Cultivation of Azotobacter.

Reference: Percival (1920 p. 181).

2483. Makrinoff's Leaf Infusion Gypsum Block

Constituents:

1. Distilled water	1000.0 cc.
2. Dry leaves	20.0 g.
3. NaCl	2.0 g.
4. K_2HPO_4	1.0 g.
5. $MgSO_4$	0.5 g.
6. $FeSO_4$	0.4 g.
7. Gypsum	300.0 g.
8. $MgCO_3$	30.0 g.
9. $MgNH_4PO_4$	3.0 g.

Preparation:

(1) Rub dry leaves until they are quite fine.

(2) Add 2.0% of (1) to distilled water and heat in the autoclave for 15 minutes at 115°C., or in streaming steam for an hour.

(3) Filter.

(4) To one liter of the filtrate add 2.0 g. NaCl, 1.0 g. K_2HPO_4, 0.5 g. $MgSO_4$ and 0.4 g. $FeSO_4$.

(5) To prepare plates mix 300.0 g. gypsum, 30.0 g. $MgCO_3$ and 3.0 g. $MgNH_4PO_4$ thoroly.

(6) Add (4) to (5) until a homogeneous half liquid mass is formed.

(7) Prepare small paper molds about 3.0 cm. high and 8 or 9 cm. in diameter. Place these molds on glass and pour (6) into them. When they have solidified level off the upper surface and remove the paper from the discs.

(8) Place the small discs in Petri dishes and add (4) to the Petri dish until the discs are nearly 2/3 immersed. Place the smooth side up and inoculations are made upon it.

(9) For the preparation of tubes mix only 40.0 g. of gypsum with the $MgCO_3$ and $MgNH_4PO_4$ instead of 300.0 g. as in (5).

(10) Continue as above only pour the mixture on glass and when it has nearly solidified cut into strips.

(11) Place the strips in test tubes, add the solution as above.

Sterilization: Sterilize for 30 minutes at 120°C.

Use: Cultivation of nitrite formers. The author reported that nitrite formers showed increased growth in presence of small amount of leaf or soil infusion.

Reference: Makrinoff (1909 p. 419).

2484. Makrinoff's Soil Infusion Gypsum Block

Same as medium 2483 but treat 16.0 to 25.0% dry soil rich in humus in the same manner as the leaves, and use instead of the leaves.

2485. Omelianski and Makrinoff's Soil Infusion Gypsum Block (Löhnis)

Constituents:

1. Water	1000.0 cc.
2. Soil	250.0 g.
3. Gypsum	300.0 g.
4. $MgCO_3$	3.0 g.
5. $MgNH_4PO_4$	3.0 g.
6. $FeSO_4$	0.4 g.
7. K_2HPO_4	0.5 g.
8. $MgSO_4$	0.3 g.
9. NaCl	0.5 g.

Preparation:

(1) Mix 300.0 g. gypsum, 3.0 g. $MgCO_3$ and 3.0 g. $MgNH_4PO_4$ carefully.

(2) Prepare a soil infusion from 250.0 g. of soil rich in humus and a liter of tap water. Do not filter.

(3) Moisten (1) with (2) and stir the whole into a pasty mass.

(4) Spread (3) on a glass plate in a layer about 0.5 to 0.75 cm. thick.

(5) Cut suitable slices to go into Petri dishes, using a glass dish, or into strips to be placed in tubes using a knife.

(6) Dissolve 6, 7, 8 and 9 in a liter of distilled water.

(7) Moisten the sterile discs or strips of (5) from the bottom with sterile (6).

Sterilization: Sterilize (5) in the hot air oven. Sterilize (6) method not given.

Use: Cultivation of nitrate bacteria.

Reference: Löhnis (1913 p. 110).

GROUP VII. MEDIA INITIALLY SOLID

A_1. Solid substratum organic
Subgroup VII-A (Med. 2486–2516)
A_2. Solid substratum inorganic
Subgroup VII-B (Med. 2517–2543)

SUBGROUP VII-A

Initially Solid Organic Substrata

Basal or Complete Media Containing Initially Solid Substrata of Plant or Animal Origin

A_1. Solid substratum of plant origin.
B_1. The choice of a variety of plant tissues or derivatives given.
Thoinot and Masselin's Vegetable Medium........................ 2486
Smith's Vegetable Medium......... 2487
Reed and Cooley's Plant Material Medium........................ 2488
Smith's Plant Material Medium.... 2489
Harrison's Vegetable Medium...... 2490
B_2. The use of a particular plant tissue or derivative specified.
C_1. Tubers or roots used.
D_1. Potatoes employed.
E_1. No additional organic materials added.
Simmonds' Shellaced Potato....... 2491
Krannhals' Sliced Potato........... 2492
Wurtz's Potato Medium............ 2493
Wurtz's Potato Slants.............. 2494
Smith's Mashed Potato Medium... 2495
E_2. Containing added organic materials.
Wurtz's Glycerol Potato........... 2496
Dalimier and Lancereaux' Opsine Bouillon Potato Medium......... 2497
Thoinot and Masselin's Malt Potato Slant........................... 2498
Peklo's Malt Extract Potato Medium.......................... 2499
Harras' Tissue Potato Medium..... 2500
D_2. Other tubers or roots employed.
Matzuschita's Carrot Medium..... 2501
Smith's Beet Medium.............. 2502
Smith's Onion Medium............. 2503
Roger's Artichoke Medium......... 2504
C_2. Fruits used.
Smith's Cocoanut Medium......... 2505
Smith's Orange Medium............ 2506
Duval's Basal Banana Medium..... 2507
C_3. Bread, macaroni, noodles, etc. used.
Heinemann's Bread Paste Medium. 2508
Logerheim's Macaroni Medium..... 2509
Logerheim's Noodle Medium....... 2510
C_4. Filter paper used.
Löhnis Filter Paper Medium...... 2511
Cunningham's Nitrate Filter Paper Medium......................... 2512
Malenkovic's Nitrate Sawdust Medium........................... 2513
A_2. Solid substratum of animal origin.
B_1. Animal tissue without other materials.
Besson's Animal Organ Medium.... 2514
B_2. Animal organs or tissue with other materials.
Ficker's Glycerol Tissue Medium... 2515
Frugoni's Glycerol Bouillon Tissue Medium (Abel).................. 2516

2486. Thoinot and Masselin's Vegetable Medium

Constituents: 1. Potato or other vegetable.

Preparation:

(1) Wash good sound potatoes thoroly.
(2) Soak for an hour in a 1 to 1000 solution of sublimate.
(3) Wash a large crystallizing dish with a 1 to 1000 sublimate solution.
(4) Place a filter paper moistened with the sublimate solution in the bottom of the dish.
(5) Cut the potato into two equal halves, using a sterile knife.
(6) Place each half in the crystallizing dish prepared as indicated above. Put the cover in place.
(7) The potatoes may be cut into discs and placed in Petri dishes.

Sterilization: Autoclave at 100°C. for one hour and then at 115° for 15 minutes at least.

Use: General culture medium.

Variants: The authors prepared turnips, black radishes or carrots in the same manner as potatoes.

Reference: Thoinot and Masselin (1902 pp. 48, 81).

2487. Smith's Vegetable Medium

Constituents: 1. Potato or other vegetable.

Preparation:

(1) Prepare slant cylinders of one of the following:

Potato
sweet potato
carrot
sugar beet
turnip
radish
salsify
parsnip
onion
banana
cocoanut
peanuts
brazilnuts
apple
pear
quince
pineapple

(2) Place in test tubes and half cover by the addition of distilled water. The pieces of vegetable may be placed in Roux tubes, or tubes containing a wad of cotton.

Sterilization: Sterilize on each of 3 successive days for 20 minutes in streaming steam.

Use: Cultivation of plant parasites.

Reference: Smith (1905 p. 40).

2488. Reed and Cooley's Plant Material Medium

Constituents:

1. Water.
2. Corn meal or other plant derivative.

Preparation:

(1) Place corn meal in test tubes.
(2) Add water.
(3) Cook in the moist heat of the sterilizer.

Sterilization: See (3) above.

Use: Cultivation of *Heterosporium variabile*.

Variants: The authors substituted rice, shredded cocoanut, or spinach leaves for corn meal.

Reference: Reed and Cooley (1911–12 p. 50).

2489. Smith's Plant Material Medium

Constituents:

1. Radish,
horse radish,
yellow turnip.
white turnip,
cabbage leaves or
cauliflower.

Preparation:

(1) Take slices of radish, horse radish roots, yellow and white turnip, or the base of cabbage or cauliflower leaves, and place in sterile test tubes.
(2) Nearly cover with distilled water.

Sterilization: Steam on 3 successive days for a few minutes.

Use: Cultivation of *Pseudomonas campestris* (Pammel). Author reported that growth was prompt and abundant on all material except horse radish. Growth on horse radish slow but finally abundant. Growth yellow in color.

Reference: Smith (1897 p. 481).

2490. Harrison's Vegetable Medium

Constituents: 1. Potato or other vegetable.

Preparation:

(1) Select sound specimens of average size of one of the following vegetables: potato, Jerusalem artichoke, cucumber, red carrot, horse radish (edible portion), radish, red beet, sugar beet, parsnip, cauliflower, cabbage, celery, mangelwurzel, Swede turnip, white turnip, kohlrabi, salsify, green tomato, ripe tomato, onion.
(2) In case of roots, wash and scrub in running water, soak for an hour in sublimate 1:1000.
(3) Cut thick slices 20–40 mm. thick with sterile knives and place in deep Petri dishes at the bottom of which is a filter paper saturated with bichloride of mercury solution (1–5000).
(4) In case of celery, cabbage, cauliflower, etc., wash the whole plant thoroly and dry.
(5) Cut down the middle with a sterilized knife.
(6) Place in large double dishes.

Sterilization: Not specified.

Use: Cultivation of *Bacillus solanisaprus* (causing bacterial rot in potatoes). Author reported that generally the bacillus developed on the vegetables listed.

Reference: Harrison (1907 pp. 172, 384).

2491. Simmonds' Shellaced Potato

Constituents:
1. Potatoes.
2. Shellac.

Preparation:
(1) Clean and boil potatoes in the usual way.
(2) Cool and tie strings on the potatoes.
(3) Dip them in a shellac solution three different times, allowing them to dry about ½ hour between each time.
(4) They are dry after about an hour and may be stored in piles.

Use: Preservation of potatoes. Author reported that potatoes treated in this manner do not lose their moisture. Might be used for anaerobic growth.

Reference: Simmonds (1897 p. 100).

2492. Krannhals' Sliced Potato

Constituents: 1. Potato.

Preparation:
(1) Wash potatoes with water and soap with a brush.
(2) Allow them to remain in a sublimate solution for a short time and rinse in boiled water.
(3) Boil the potatoes or heat about 2 hours in streaming steam.
(4) Cut in thin slices, about 1 cm. thick, with a sterilized knife.
(5) Place 4 to 6 slices in a flat dish covered with a projecting covering (Petri dish ?). This dish contains a layer of hygroscopic cotton which has been saturated with 1.0% sublimate solution and then carefully pressed out. A piece of blotting paper or a layer of cotton soaked up with sterile distilled water may also be added if incubated in the incubator.
(6) Add either drop by drop 1 to 2% aqueous solution of sodium bicarbonate on the side until a noticeable adsorption has taken place or pour several cc. of soda solution on the bottom of the plate so that the plate is scarcely covered.

Sterilization: Place the plates for 30 minutes in streaming steam on 3 or 4 successive days.

Use: Cultivation of comma bacilli. Other investigators used a medium similarly prepared to cultivate a large variety of organisms.

Variants:
(a) The author reported that medium may be used without the addition of soda solution or sodium bicarbonate.
(b) Migula prepared the potatoes as follows:
  (1) Peel good sound potatoes with few eyes and cut in discs one centimeter thick.
  (2) Wash repeatedly in water.
  (3) Place in Petri dishes.
  (4) Boil for one hour in the steamer.
  (5) On the following day steam for 30 minutes.
  (6) Potatoes may be cut in cylindrical pieces and placed in tubes containing a little water and some cotton in the bottom of the tube.
  (7) If the potatoes do not react slightly acid, add a drop or two of 0.5% citric acid to the surface of the potato discs or cylinders.
(c) Various investigators prepared potatoes in essentially the same manner. The methods employed were not sufficiently different to those given to warrant individual mention.

References: Krannhals (1893 p. 37), Migula (1901 p. 22), Abel (1912 p. 28), Besson (1920 p. 56), Abbott (1921 p. 132), Harvey (1921–22 p. 118), Klimmer (1923 p. 205).

2493. Wurtz's Potato Medium

Constituents: 1. Potato.

Preparation:
(1) Thoroly wash sound potatoes.
(2) Place in a 1 to 1000 sublimate solution for one hour.
(3) Wash crystallizing dishes and filter papers in a 1:1000 sublimate solution.
(4) Cut the potatoes in half, and put each half in one of the crystallizing dishes containing a filter paper.

Sterilization: Cover the dishes and autoclave at 125°C. for 30 minutes.

Use: General culture medium.

Variants:
(a) Smith prepared the potatoes as follows:
  (1) Wash large sound potatoes in running water, using a stiff brush.

(2) Remove all the eyes and any suspicious spots.
(3) Soak in 1:1000 solution of bichloride of mercury for an hour.
(4) Steam at 100°C. for one hour.
(5) Do not remove from the sterilizer but steam for 15 minutes on the second and third days.
(6) Divide the potatoes in halves by means of a sterilized knife, and place in a sterilized container (Petri dish) with the cut surfaces uppermost. Do this under aseptic conditions.

(b) A number of different investigators prepared potatoes in a very similar manner. The individual differences were not large enough to warrant a discussion of each method.

References: Wurtz (1897 p. 31), Smith (1902 p. 74), Abel (1912 p. 27), Tausz and Peter (1919 p. 49), Klimmer (1923 p. 204).

2494. Wurtz's Potato Slants

Constituents:
1. Potato.

Preparation:
(1) Cut well washed potatoes in rectangular prisms 5 cm. long.
(2) Place in Roux tubes.

Sterilization: Sterilize at 120° for 30 minutes.

Use: General culture medium.

Variants:

(a) Thoinot and Masselin prepared the potatoes as follows:
(1) Cut clean potatoes into rectangular pieces or prepare cylindrical pieces by use of a cork borer.
(2) Split the cylinders giving semi-cylinders.
(3) Wash the pieces of potato carefully with water and dry between filter paper.
(4) Place each piece in a Roux tube.
(5) Sterilize in the autoclave at 115° for 30 minutes, or at 120° for 20 minutes.

(b) Smith prepared the medium as follows:
(1) Wash large sound potatoes in running water using a stiff brush.
(2) Remove all the eyes and any suspicious spots.
(3) Cut the ends from the potatoes and cut cylindrical pieces, using a cork borer.
(4) Cut the cylinders obliquely so that each resulting piece presents a round flat end and a large oval beveled surface.
(5) Place the pieces in running water for several hours or over night if convenient.
(6) Place one piece of potato into sterile tubes containing a bit of glass rod. The beveled side of the potato should be up.
(7) Sterilize by heating at 100°C. for 30 minutes on one day and for 15 minutes on the second and third successive days, or in the autoclave at 120°C. or 30 to 40 minutes.
(8) The potatoes may be cut in discs, placed in Petri dishes and then sterilized.

(c) Roddy used the following method to prepare potatoes:
(1) Wash a smooth new potato in hot water, hot 1:500 bichloride and then in several changes of hot water.
(2) Cut cylindrical pieces about 1.5 inches long and about the same diameter of the tubes in which they are to be placed.
(3) Split each cylinder diagonally in two thru the long axis.
(4) Place each piece in a tube, with the thick end at the bottom.
(5) Add about 0.25 inch of distilled water.
(6) Sterilize in the autoclave.

(d) Harvey gave the following method of preparation:
(1) Wash and scrub the potato thoroly.
(2) Peel and remove eyes.
(3) Prepare cylinders by means of an apple corer or a large cork borer.
(4) Divide the cylinder diagonally in two parts.
(5) Soak for not more than 24 hours in 1–1000 sodium carbonate.
(6) Place on top of sterilized cotton wool moistened with sterile water in the bottom of a tube.
(7) Sterilize.

(e) Pitfield gave the following method of preparation:
  (1) Cut semi-cylinders from well washed large potatoes using a Ravenel potato cutter.
  (2) Wash the potato semi-cylinders in a 1:10,000 bichloride of mercury solution and then in running water over night.
  (3) Thrust cotton in the bottom of a tube and wet with distilled water.
  (4) Place the potato cylinders on the cotton.
  (5) Plug.
  (6) Sterilize in the autoclave (time or temperature not specified).
(f) Other investigators prepared potato slants in essentially the same manner. Their methods were not sufficiently different to warrant an individual discussion.

References: Wurtz (1897 p. 32), Thoinot and Masselin (1902 p. 50), Smith (1902 p. 75), Frost (1903 p. 18), Heinemann (1905 p. 27), Roux and Rochaix (1911 p. 120), Abel (1912 p. 28), Löhnis (1913 p. 20), Roddy (1917 p. 46), Ball (1919 pp. 72, 73), Percival (1920 p. 58), Bezançon (1920 p. 114), Besson (1920 p. 56), Roux and Rochaix (1921 p. 121), Dopter and Sacquépée (1921 p. 128), Giltner (1921 p. 26), Abbott (1921 p. 132), Harvey (1921–22 p. 118), Pitfield (1922 p. 117), Stitt (1923 p. 41), Klimmer (1923 p. 205), Park, Williams and Krumwiede (1924 p. 121), Cunningham (1924 p. 17).

2495. Smith's Mashed Potato Medium

Constituents:
1. Potato.

Preparation:
(1) Mash thoroly cleaned and boiled potatoes.
(2) Spread in Petri dishes or tube.

Sterilization: Method not given.

Use: Owen cultivated *B. saccharalis* on mashed potatoes.

Variants:
(a) Abel crushed boiled potatoes to a pulp adding a little water or milk, and sterilized in a steamer.
(b) Ball prepared a similar medium as follows:
  (1) Mash peeled potatoes with distilled water until thick.
  (2) Sterilize in flasks for 45 minutes on 3 successive days.
(c) Tanner gave the following method of preparation:
  (1) Clean large potatoes and cut into slices.
  (2) Boil until soft.
  (3) Grate, or press thru ricer.
  (4) Distribute in layers in Petri dishes.
  (5) Sterilize at 120° for 20 minutes.
  (6) The slices may be placed in the Petri dishes without mashing if desired.
(d) Bushnell used the following medium to study chemical changes produced by the *B. sporogenes* group:
  (1) Peel potato and pass thru meat grinder. Wash ground potato in running water several hours.
  (2) Sterilize at 20 pounds for 30 minutes.
  (3) No adjustment of reaction given.

References: Smith (1902 p. 76), Owen (1916 p. 243), Abel (1912 p. 28), Ball (1919 p. 73), Tanner (1919 p. 60), Besson (1920 p. 57), Dopter and Sacquépée (1921 p. 129), Abbott (1921 p. 132), Harvey (1921–22 p. 119), Bushnell (1922 p. 381).

2496. Wurtz's Glycerol Potato

Constituents:
1. Potato.
2. Glycerol (8.0%).

Preparation: (1) Add sufficient sterile 8.0% glycerol to a Roux tube containing a piece of potato, so that the bottom of the potato slant is immersed in the glycerol.

Sterilization: Method not given.

Use: Cultivation of tubercle bacilli. Peklo cultivated plant actinomyces on a similar medium.

Variants: The following investigators have prepared glycerol potato as indicated.
(a) Matzuschita (1899).
  (1) Cut pieces of potato with a cylindrical borer.
  (2) Cut away the end portion.
  (3) Halve the cylindrical pieces of potato and place in plates, containing glycerol (or water). The pieces may have been soaked in 1.0% soda solution for 15 minutes.

  - (4) Sterilize 3 times for 1.2 hour each time (exact method not given).
- (b) Thoinot and Masselin (1902).
  - (1) Sterilize pieces of potato properly prepared in Roux tubes.
  - (2) Add 4.0% glycerol in distilled water or bouillon to each tube to fill the tube to the constriction in the tube.
  - (3) After inoculation slant the tubes so that the glycerol is in contact with the potato.
- (c) Smith (1902).
  - (1) Wash large sound potatoes in running water using a stiff brush.
  - (2) Remove all the eyes and any suspicious spots.
  - (3) Cut the ends from the potatoes and cut cylindrical pieces using a cork borer.
  - (4) Cut the cylinders obliquely so that each resulting piece presents a round flat end and a large oval beveled surface.
  - (5) Place the pieces in running water for several hours or over night if convenient.
  - (6) Place one piece of potato into sterile tubes containing a bit of glass rod. The beveled side of the potato should be up.
  - (7) Add a 5.0% glycerol solution up to the level of the lower surface of the potato.
  - (8) Sterilize by heating at 100°C. for 30 minutes on one day and for 15 minutes on the second and third successive days, or in the autoclave at 120°C. for 30 to 40 minutes.
- (d) Heinemann (1905).
  - (1) Prepare potato slants in the ordinary manner.
  - (2) Soak the potato slants in 25.0% solution of glycerol.
  - (3) Add a little glycerol solution to the bottom of the tube.
- (e) Anzilotti (1906).
  - (1) Wash sound potatoes.
  - (2) Cut cylindrical pieces from the potatoes using a cork borer.
  - (3) Split the cylinders, and wash the potatoes carefully in distilled water.
  - (4) Boil the potatoes in a 6.0% glycerol solution that has been made alkaline by the addition of concentrated solution of Na_2CO_3. Boil until the potatoes have become soft and swollen usually requires 20 minutes. It is necessary to keep the reaction alkaline by the addition of more Na_2CO_3 solution.
  - (5) Place the pieces of potato into sterile Roux tubes, or a piece of glass tubing may be placed in an ordinary tube so that the potato will be about 2 mm. from the bottom of the tube.
  - (6) Pour the alkaline 6.0% glycerol solution into the tubes so that the bottom of the potato is covered with the solution.
  - (7) Plug the tubes with cotton.
  - (8) Steam on 3 successive days for 20 minutes in a Koch apparatus, or sterilize for 20 minutes in an autoclave at 120°C.
- (f) Peklo impregnated potato slants with 3.0% glycerol solution.
- (g) Park and Krumwiede (1910).
  - (1) Cut potatoes in usual Bolton method (Details of method not specified).
  - (2) Soak in 1–1000 sodium carbonate solution in distilled water for 24 hours.
  - (3) Drain.
  - (4) Soak in 5.0% glycerol in distilled water solution for 24 hours.
  - (5) Tube, using $\frac{3}{4}$ inch tubes and add glycerol water in which they were soaked to cover about $\frac{1}{3}$ of the surface. In some instances, to obtain a large number of pellicles, 5.0% glycerol bouillon was used to cover the potato. Pellicle formation good using plain glycerol water, however.
  - (6) Sterilize by the intermittent method in the Arnold sterilizer.
  - (7) After inoculation seal the tubes either with cork alone or preferably dip the cotton stopper also in paraffin.
- (h) Nocard (Roux and Rochaix) (1911).
  - (1) Soak pieces of peeled potato in 15.0% glycerol for 48 hours.

(2) Place each piece of potato in a Roux tube.
(3) Sterilize.

(i) Roux and Rochaix (1911).
(1) Add sufficient quantity of sterile 4.0% glycerol solution to sterile potato slants so that the lower half of the potato is immersed in the glycerol solution.

(j) Anzilotti (Kolle and Wasserman) (1912).
(1) Boil potato slants in a 6.0% solution of glycerol made alkaline by the addition of Na_2CO_3. The boiling is continued until the potatoes have swollen (usually about 20 minutes). If the reaction changes, add more Na_2CO_3.
(2) Place the slants so prepared in Roux tubes, containing a little 6.0% glycerol bouillon.
(3) Sterilize at 120°C. for 20 minutes

(k) Harvey (1921–22).
(1) Cover a half cylinder of potato with 6.0% glycerol.
(2) Steam 30 minutes.
(3) Discard the fluid covering the potato.
(4) Sterilize 30 minutes at 100°C.

(l) Harvey (1921–22).
(1) Scrub a number of potatoes of unbroken skin thoroly with a stiff brush.
(2) Cut off the outer skin.
(3) Cut out of the potato with a potato borer a cylinder of about 3 to 4 inches in diameter.
(4) Divide the cylinder diagonally into two parts.
(5) Wash in running water over night.
(6) Soak the cut out cylinder in 6.0% glycerol and soak the wool in the test tube also in 6.0% glycerol.
(7) Sterilize 20 minutes at 120°C.

(m) Harvey (1921–22).
(1) Soak diagonally cut cylinders of potato in 1–1000 sodium carbonate 24 hours.
(2) Transfer to 5.0% glycerol 24 hours.
(3) Distribute in test tubes containing a pledget of wool at the bottom.
(4) Fill up the tubes with the 5.0% glycerol.
(5) Sterilize 3 days at 100°C.
(6) Pour off the glycerol at the time of use.

(n) Dopter and Sacquépée (1921).
(1) Scrub large white whole potatoes in water.
(2) Cut cylindrical pieces from the potato using a cork borer.
(3) Allow the potatoes to soak in a 6 to 15 part per 100 glycerol solution for 48 hours.
(4) Add glycerol solution to Roux tubes until the solution is level with the constriction.
(5) Place a cylinder of potato in each. tube.
(6) Sterilize (method not given).

(o) Klimmer (1923).
(1) Thoroly wash and scrub medium sized, sound unimpaired potatoes with a scrubbing brush, soap and water.
(2) Carefully remove all eyes and scabs.
(3) Soak the potatoes for 1 to 2 hours in a 1.0% sublimate solution.
(4) Make the potato slants slightly alkaline using soda solution.
(5) Place each piece of potato in a Roux tube.
(6) Add 5.0 to 6.0% glycerol solution to each tube until half the potato is immersed in the liquid.
(7) Cook for about 20 minutes in the steamer until the potato is soft.
(8) Sterilize for 45 minutes in the steamer.

(p) Cunningham (1924).
(1) Place a small piece of cotton-wool in the bottom of each of a number of dry test tubes.
(2) Moisten each plug with 2 or 3 drops of water (not more).
(3) Scrub several sound potatoes under the tap and peel carefully.
(4) Cut into pieces about 1 x 1 x 4 to 5 cm.
(5) Divide each piece into large wedge-shaped portions by cutting the pieces diagonally.
(6) Drop each piece as it is cut into a beaker of clean water.
(7) Cover with 6.0% glycerol in distilled water in a beaker.
(8) Steam for 30 minutes.

(9) Pour off the glycerol and place one of the wedges in each of the prepared tubes. The broad end of the wedge at the bottom.

(10) Sterilize in the autoclave at 22.5 pounds pressure.

(q) Park, Williams and Krumwiede (1924).

(1) Scrub large white potato under running water.

(2) Cut cylinders by means of an apple corer.

(3) Cut the skin off the ends of the potato cylinders.

(4) Make 2 wedge shapes from each cylinder by cutting thru obliquely.

(5) Soak the potatoes over night in a 1:1000 Na_2CO_3 solution.

(6) Drain.

(7) Cover with a 5.0% glycerol solution for 24 hours.

(8) Place the pieces in test tubes one inch or more in diameter.

(9) Add a little of the glycerol solution, or water to each tube.

(10) Sterilize in the autoclave at 15 pounds pressure for 30 minutes.

References: Wurtz (1897 p. 46), Matzuschita (1899 p. 128), Thoinot and Masselin (1902 p. 53), Smith (1902 p. 78), Heinemann (1905 p. 129), Anzilotti (1906 p. 766), Peklo (1910 p. 473), Park and Krumwiede (1910 p. 215), Roux and Rochaix (1911 p. 122), Kolle and Wasserman (1912 p. 408), Harvey (1921–22 p. 118), Dopter and Sacquépée (1921 p. 129), Klimmer (1923 p. 206), Cunningham (1924 p. 166), Park, Williams and Krumwiede (1924 p. 122).

2497. Dalimier and Lancereaux' Opsine Bouillon Potato Medium

Constituents:

1. Bouillon.
2. Opsine.
3. Potato.
4. Glycerol.

Preparation:

(1) Prepare bouillon with a slightly alkaline reaction.

(2) Add opsine (a commercial mixture of amino acid) and glycerol (amount not given to (1).

(3) Place potato slants in Roux tubes.

(4) Pour (2) over (3).

Sterilization: Not specified.

Use: Cultivation of tubercle bacilli and other saprophytic and parasitic forms.

Reference: Dalimier and Lancereaux (1913 p. 419).

2498. Thoinot and Masselin's Malt Potato Slant

Constituents:

1. Water	100.0 cc.
2. Malt dust	10.0 g.
3. Sucrose	5.0 g.
4. Glycerol	2.0 g.
5. Potato	

Preparation:

(1) Prepare an infusion from 10 parts malt dust and 100 parts water.

(2) Add 5 parts sucrose and 2 parts glycerol to (1).

(3) Prepare potato slants in Roux tubes.

(4) Add (2) to each tube of sterile (3) to fill the tube to the constriction in the tube.

(5) Slant the tube after inoculation so as to have the potato in contact with the liquid.

Sterilization: Method of sterilization of potato slant not given. Sterilization of (2) not specified.

Use: Cultivation of streptothrix, tubercle bacilli and molds.

Reference: Thoinot and Masselin (1902 p. 52).

2499. Peklo's Malt Extract Medium

Constituents:

1. Distilled water	
2. Malt	
3. K_2HPO_4	8.0 g.
4. K_2CO_3	6.0 g.
5. Potato	

Preparation:

(1) Thoroly mix malt and distilled water.

(2) Boil for 1 hour and allow to stand at 60°C. for 24 hours.

(3) Boil again for one hour.

(4) Filter.

(5) Dilute the filtrate with distilled water (amount not given).

(6) To 700.0 cc. of sterile (5) add 780.0 cc. distilled water, 8.0 g. K_2HPO_4 and 6.0 g. K_2CO_3.

(7) Impregnate potato slants with sterile (6).

Sterilization: Sterilize (5) by heating for several successive days and filter after each heating. Sterilization of potato slants not specified. Sterilize (7) method not given.

Use: Cultivation of plant actinomyces.

Reference: Peklo (1910 p. 473).

2500. Harrass' Tissue Potato Medium

Constituents:
1. Potato.
2. Liver.

Preparation:
(1) Grind brain or liver in a meat chopping machine.
(2) Peel well brushed potatoes and cut into small cubes.
(3) Cook the potato cubes well in water.
(4) Add brain or liver pulp to the well-cooked potato cubes and pound to a pulp.
(5) Distribute in Petri dishes.

Sterilization: Sterilize 1.5 to 2 hours in streaming steam. If the consistency is too thin place in a drying incubator until the desired consistency is reached.

Use: Cultivation of obligate anaerobes.

Variants: Author used brain instead of liver.

Reference: Harrass (1906 p. 2237).

2501. Matzuschita's Carrot Medium

Constituent:
1. Carrot.

Preparation:
(1) Shove a cylindrical borer thru a carrot.
(2) Cut away the end portions of the cylindrical piece of carrot.
(3) Halve the cylindrical piece of carrot.
(4) Place in plates containing water.

Sterilization: Sterilize on each of 3 successive days for 30 minutes in the steamer.

Use: Cultivation of tubercle bacilli, mammalian and chicken. Other investigators used similar media for a variety of purposes.

Variants:
(a) The author specified that carrot may be soaked in a 1.0% watery solution of soda for ½ hour before sterilizing. This gives an alkaline carrot. Growth generally more luxuriant on alkaline medium.
(b) The author used glycerol in the plates containing the carrot instead of water as indicated in step (4) above.
(c) Roux and Rochaix prepared carrot in exactly the same manner as potato. See variant (h) and (i), medium 2496 and medium 91.
(d) Dopter and Sacquépée gave the following method of preparation.
  (1) Scrub large whole carrots with water.
  (2) Cut cylindrical pieces.
  (3) Wash the cylinders thoroly with water.
  (4) Dry on filter paper.
  (5) Place in Roux tubes.
  (6) Sterilize at 120° for 20 minutes.
  (7) The carrots may be cut in discs, placed in Petri dishes and sterilized.
(e) Davis used sterilized sliced carrots to demonstrate chromogenesis. He reported that a deep shiny black pigment was formed that did not diffuse into the carrot.

References: Matzuschita (1899 p. 128), Roux and Rochaix (1911 p. 123), Dopter and Sacquépée (1911 p. 129), Davis (1915 p. 179).

2502. Smith's Beet Medium

Constituents:
1. Beet.

Preparation:
(1) Pare and cut small red turnip-beets into long slices and place in sterile test tubes.
(2) Nearly cover with distilled water.

Sterilization: Steam on 3 successive days for 20 to 30 minutes.

Use: Cultivation of *Pseudomonas campestris* (Pammel). Author reported that on the surface in the air organism made a prompt and copious pale yellow growth, while the fluid was changed to its original bright yellow color. Matzuschita cultivated mammalian and chicken tubercle bacilli on a similar medium.

Variants: Matzuschita prepared the medium as follows:
(1) Shove a cylindrical borer thru a beet.
(2) Cut away the end portions of the cylindrical piece of beet.

(3) Halve the cylindrical piece of beet.
(4) Place in plates containing glycerol (or water). (The pieces may have been previously soaked in 1.0% soda solution for 15 minutes.)
(5) Sterilize in the steamer on each of 3 successive days for 30 minutes.

References: Smith (1897 p. 481), Matzuschita (1899 p. 129).

2503. Smith's Onion Medium

Constituents:
1. Onion.

Preparation:
(1) Slice a sweet Bermuda onion and place in test tubes.
(2) Nearly cover the onion with distilled water.

Sterilization: Steam for a few minutes on 3 successive days.

Use: Cultivation of *Pseudomonas campestris* (Pammel). Author reported that organism formed a pale yellow slime and a rather copious pale precipitate.

Reference: Smith (1897 p. 482).

2504. Roger's Artichoke Medium

Constituents:
1. Artichoke.

Preparation:
(1) Wash the leaves of artichoke, and cut the bottom into small pieces.
(2) Place the small pieces, the leaves on top into tubes, whose ends are plugged with moist cotton.

Sterilization: Sterilize at 115° for 15 minutes.

Use: Cultivation of streptococci, diphtheria bacilli, *Bacillus subtilis*, *Bacillus prodigiosus* and others.

Variants:
(a) Roux and Rochaix sterilized at 115°C. for 20 minutes.
(b) Besson added a little water to each tube.
(c) Bezançon sterilized at 175°C., time not given.

References: Roger (1898 p. 769), Roux and Rochaix (1911 p. 123), Besson (1920 p. 51), Bezançon (1920 p. 115), Dopter and Sacquépée (1921 p. 129).

2505. Smith's Coconut Medium

Constituents:
1. Coconut.

Preparation:
(1) Cut the flesh of a coconut into strips.
(2) Place the strips into sterile test tubes.

Sterilization: Steam on 3 successive days for a few minutes.

Use: Cultivation of *Pseudomonas campestris* (Pammel). Author reported that the organism produced a pale yellow growth on the surface. Schouten cultivated fungi and yeast on coconut.

References: Smith (1897 p. 481), Schouten (1916 p. 480).

2506. Smith's Orange Medium

Constituents:
1. Orange.

Preparation:
(1) Segment a rather tart but palatable California Navel orange.
(2) Place into sterile cotton plugged test tubes and cover with distilled water.

Sterilization: Steam on 3 successive days for several minutes.

Use: Cultivation of *Pseudomonas campestris* (Pammel). Author reported no growth.

Reference: Smith (1897 p. 480).

2507. Duval's Basal Banana Medium

Constituents:
1. Banana.

Preparation:
(1) Carefully remove the rind from the fruit portion of fully matured green bananas, using every caution to avoid contamination.
(2) Slant one surface of the fruit with a sterile sharp knife and place into sterile glass cylinders containing cotton plugs saturated with sterile distilled water.
(3) Prepare 1.0% solutions of one of the added nutrients.
(4) Add one of sterile (3) to the surface of the banana.

Sterilization: Sterilize (3) by passing thru a Berkefeld.

Use: Cultivation of *B. leprae*. Author reported that cystein gave excellent results.

Added nutrients: The author used a 1.0% solution of tryptophane, cystein (prepared from protein) or leucine.

Variants: The author used the added nutrients in combination.

Reference: Duval (1910 p. 655).

2508. Heinemann's Bread Paste Medium

Constituents:
1. Water.
2. Bread.

Preparation:
(1) Cut bread into slices.
(2) Dry in the oven.
(3) Pulverize.
(4) Distribute in 100.0 cc. flasks until the layer on the bottom is half an inch thick.
(5) Gradually run water in to cover the surface of the bread.

Sterilization: Sterilize in the Arnold.

Use: Cultivation of molds and bacteria.

Variants:
(a) Tanner soaked slices of bread in distilled water and sterilized at 115°C.
(b) Tanner added 2.5 parts of water to 1 part by weight of ground bread crumbs and sterilized the mixture at 115°C. for 20 minutes.
(c) Ball sterilized a paste prepared from bread crumbs and water in the steamer on each of 3 successive days for 45 minutes.
(d) Lloyd, Clark and McCrea cultivated Flügge's Mesentericus group on a medium prepared as follows:
 (1) Cut small pieces of bread a quarter of an inch thick and sterilize them in Petri dishes in the autoclave for 20 minutes at 115°C.

References: Heinemann (1905 p. 128), Abel (1912 p. 29), Löhnis (1913 p. 88), Tanner (1919 p. 59), Ball (1919 p. 73), Besson (1920 p. 58), Lloyd and McCrea (1920–21 p. 383), Harvey (1921–22 p. 120), Klimmer (1923 p. 207).

2509. Logerheim's Macaroni Medium

Constituents:
1. Macaroni.

Preparation:
(1) Secure white macaroni about 5.0 mm. in thickness with a 3.0 mm. caliber.
(2) Break in pieces about 4.5 cm. long and place in sterile test tubes.
(3) Fill the tubes with water so that the macaroni is immersed and about 1.0 cm. of water above the macaroni.
(4) Boil until the macaroni swells (requires about 15 minutes).
(5) Pour off all the water and plug the tubes.

Sterilization: Sterilize in the usual way in the steamer.

Use: Cultivation of chromogenic and other bacteria. Author reported the medium as being a good substitute for potato. May be used in Petri dishes instead of tubes.

Reference: Logerheim (1892 p. 147).

2510. Logerheim's Noodle Medium

Treat noodles in exactly the same manner as macaroni in preceding medium 2509.

2511. Löhnis Filter Paper Medium

Constituents:
1. Filter paper.
2. $MgNH_4PO_4$.
3. K_2HPO_4 (0.05% soln.).

Preparation:
(1) Place two filter papers in Petri dishes.
(2) Sprinkle $MgNH_4PO_4$ between the filter papers and moisten with a 0.05% aqueous solution of K_2HPO_4.

Sterilization: Not specified.

Use: Cultivation of cellulose decomposers from the soil.

References: Löhnis (1913 p. 104), Cunningham (1924 p. 129).

2512. Cunningham's Nitrate Filter Paper Medium

Constituents:

1. Water (tap)	1000.0 cc.
2. K_2HPO_4	0.5 g.
3. NH_4NO_3	0.5 g.
4. Cellulose	

Preparation:
(1) Place two discs of filter paper in a Petri dish and add a little rich garden soil.
(2) Dissolve 2 and 3 in 1.
(3) Moisten (1) and keep moist by the addition of (2).

Sterilization: Not specified.

Use: Decomposition of cellulose by fungi.

Reference: Cunningham (1924 p. 141).

2513. Malenkovic's Nitrate Sawdust Medium

Constituents:

1. Water	1000.0 cc.
2. KNO_3	1.5 g.

3. K_2HPO_4	1.5 g.
4. $NH_4H_2PO_4$	1.0 g.
5. $MgSO_4$	0.5 g.
6. Sawdust	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.

(2) Exhaust sawdust with water, cold ammonia or boiling KOH (method not given). (When exhausting with KOH have the wood free from xylan.)

(3) Moisten (2) with (1).

Sterilization: Not specified.

Use: Cultivation of *Coniphpora cerebella* (*Corticium putaneum*). Author reported that growth was generally good.

Variants: The author prepared cellulose from beechwood according to Lange's method (by heating with potassium acetate, details of method not given), or prepared lignin acid by Lange's method, and substituted either cellulose or lignin acid for sawdust.

Reference: Malenkovic (1906 p. 412).

2514. Besson's Animal Organ Medium

Constituents:

1. Animal organs.

Preparation:

(1) Obtain the organs (placenta, liver, spleen, kidney, etc.) under as nearly aseptic conditions as possible and place in a sterile container.

(2) Sear the surface of the organs with a hot metal.

(3) Cut small pieces from the organ, using a sterilized knife and pincher.

(4) Distribute in sterile Petri dishes or large sterile test tubes.

(5) Incubate for 2 days at 37°C. to test sterility.

Sterilization: The whole method of preparation should be carried out under as nearly aseptic conditions as possible.

Use: General culture medium.

Variants:

(a) Harvey cultivated spirochetes and anaerobic organisms on organs prepared as follows:

  (1) Kill a rabbit.

  (2) Fasten it on a post-mortem board on its back with limbs fully extended and pinned to the board.

  (3) Wet the hair over thorax and abdomen with 2.0% lysol or other antiseptic.

  (4) Cut the hair as short as possible and again wet the body surface with antiseptic solution.

  (5) Make a mesial incision with sterile instruments thru the skin, to extend over thorax and abdomen.

  (6) Dissect off the skin in flaps to expose the anterior surface of thorax and abdomen completely.

  (7) Use a fresh set of sterile instruments and open the abdomen.

  (8) Remove with sterile precautions the organs, liver, kidneys, spleen and testicles.

  NOTE: Organs and tissues obtained at ordinary post mortem examination may also be used after sterilization.

  (9) Place in sterile Petri dishes.

  (10) Open the thorax with a fresh set of sterile instruments.

  NOTE: If the thorax as well as the abdomen is to be opened, the former should be opened first.

  (11) Remove the lungs and place in sterile Petri dishes.

  (12) Cut up the organs obtained with sterile precautions.

(b) Harvey removed the lungs, liver, kidney, spleen and testicles from a rabbit prepared as indicated above. Treat the organs as follows:

  (1) Sear the surface of the organs.

  (2) Cut out suitably sized portions with sterile forceps and scissors.

  (3) Place in sterile test tubes or Petri dishes.

References: Besson (1920 p. 55), Harvey (1921–22 p. 97).

2515. Ficker's Glycerol Tissue Medium

Constituents:

1. Glycerol 3.0% solution.
2. Brain.

Preparation:

(1) Place brain tissue in an empty container and steam for 60 to 90 minutes.

(2) Remove from steamer and slice into pieces.

(3) Place the pieces in plates or tubes.

(4) Add to the tube about 10 drops of a 3.0% glycerol and to the plate 15 to 20 drops.

Sterilization: Sterilize twice for 30 minutes each time in steam.

Use: Cultivation of tubercle bacilli. Author reported that human, sheep, beef, calf and horse brain were used but all gave same results.

Variants:

(a) Frugoni prepared a similar medium as follows:

(1) Place animal tissue (lung of rabbit or dog) in the autoclave for 45 minutes.
(2) Cut in small prism-like pieces under aseptic conditions from the sterile tissue.
(3) Allow the pieces to soak in 6–8.0% glycerol solution.
(4) Place the glycerolated tissue in sterile Roux tubes.
(5) Add 6–8.0% glycerol solution to the tube so that the tissue is touching the solution.

(b) Harvey gave the following method of preparation:

(1) Treat cut up portions of organs sterilely removed from a rabbit specially killed for the purpose (see variant (a) medium 2514) with 6.0% glycerolated 0.85% sterile salt solution for one hour.
(2) Prepare sterile test tubes containing a 2 cm. length of glass tubing as support for the tissue.
(3) Add 6.0% glycerolated 0.85 sterile salt solution to the test tubes to the top of the supporting glass rod.
(4) Transfer a suitably sized portion of glycerolated organ tissue to each test tube to rest on the glass tubing support.
(5) Sterilize 30 minutes at 120°C.

References: Ficker (1900 p. 593), Frugoni (1910 p. 557), Kolle and Wassermann (1912 p. 409), Harvey (1921–22 p. 98).

2516. Frugoni's Glycerol Bouillon Tissue Medium (Ficker)

Constituents:

1. Tissue (rabbit).
2. Glycerol bouillon.

Preparation:

(1) Soak fresh rabbit lung or other tissue for one hour in 0.85% NaCl solution containing 6.0% glycerol.
(2) Support sterile (1) over the surface of 6.0% glycerol bouillon so that the tissue is kept moist by capillary attraction and condensation. (Post mortem tissue may be used after sterilizing by boiling or in the autoclave.)

Sterilization: Sterilize in the autoclave at 120°C. for 30 to 45 minutes.

Use: Cultivation of tubercle bacilli.

Variants: Klimmer prepared the medium as follows:

(1) Heat the liver and lungs of a dog or rabbit in the autoclave for 30 to 45 minutes.
(2) Cut (1) into strips or prisms.
(3) Soak (2) in 6 to 8.0% glycerol solution for 1 to 2 hours.
(4) Place in containers so that the tissue is just touching 6.0 to 8.0% glycerol bouillon. It may be necessary to have the pieces of tissue rest on a piece of glass rod.

References: Ficker (1900 p. 593), Frugoni (1910 p. 557), Abel (1912 p. 98), Klimmer (1923 p. 201).

SUBGROUP VII-B

Initially Solid Inorganic Substrata

Basal or Complete Media Containing Initially Solid Inorganic Materials

A_1. Sand only employed.
B_1. Organic constituents not added.
C_1. Nitrogen supplied as ammonium salts.
Münter's Basal Ammonium Nitrate Sand Medium 2517
Gowda's Ammonium Sulphate Sand Medium 2518
Wichers' Ammonium Sulphate Sand Medium 2519
C_2. Nitrogen supplied as nitrites or nitrates.
Malenkovic's Basal Nitrate Sand Medium 2520
Stigell's Nitrate Sand Medium 2521
B_2. Organic constituents added.
C_1. All additional constituents of known chemical composition.
Krüger and Schneidewind's Basal Glucose Sand Medium 2522
Ashby's Basal Mannitol Sand Medium (Reed and Williams) 2523
Münter's Basal Carbohydrate Asparagin Sand Medium 2524

C_2. At least one additional constituent of unknown chemical composition.
Heinze's Beerwort Sand Medium ... 2525
Krüger and Schneidewind's Extract Broth Sand Medium (Heinze) 2526
Stigell's Bouillon Sand Medium 2527
Heinze's Loam Sand Medium 2528
A_2. Materials other than sand present.
B_1. A variety of "starra" (solid) substrata specified.
B_2. A single starra (solid) substratum specified.
Mortensen's "Stone" media 2529
C_1. Organic materials not added.
Wojtkiewicz's Soil Medium 2530
Makrinoff's Carbonate Soil Medium 2531
Wichers' Ammonium Sulphate Permutite Medium 2532
Rössler's Iron Sulphate Brick Medium 2533
Mortensen's Basal Nitrate "Starre" Medium 2534
C_2. Organic materials added.
D_1. All additional constituents of known chemical composition.
Greaves' Mannitol Soil Medium 2535
Vierling's Cellulose Soil Medium ... 2536
Mortensen's Ammonium Sulphate "Starre" Medium 2537
D_2. At least one additional constituent of unknown chemical composition.
E_1. Peptone added.
Zikes' Glucose Peptone Soil Medium 2538
Mortensen's Peptone "Starre" Medium 2539
E_2. Peptone not added.
Lipman's Dried Blood Soil Medium. 2540
Greaves' Dried Blood Soil Medium. 2541
Schönbrunn's Horn Meal Soil Medium 2542
Löhnis and Green's Phosphate Meal Medium 2543

2517. Münter's Basal Ammonium Nitrate Sand Medium

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4$	0.5 g.
3. NaCl	0.5 g.
4. $CaCl_2$	0.2 g.
5. KH_2PO_4	1.5 g.
6. NH_4NO_3	1.5 g.
7. $FeCl_3$	trace
8. Sand	

Preparation:
(1) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(2) Make slightly alkaline by the addition of Na_2CO_3.
(3) Distribute in 100.0 cc. lots.
(4) Dissolve 0.5% of one of the added nutrients in (3).
(5) Add 12.0 cc. of (4) to 50.0 g. of sand.

Sterilization: Not specified.

Use: Cultivation of *Actinomyces odorifer. Act. chromogenes, Act. albus I* and *II, Act. S. a. b.* and *c.*

Added nutrients: The author added 0.5% of one of the following: urea, sulphocarbamide, alanin, tyrosin, dicyandiamide.

Variants: Münter gave the following variants:
(a) Used 1.0 g. K_2HPO_4 instead of 1.5 K_2HPO_4, omitted the trace of $FeCl_3$, dissolved the salts in 500.0 cc. instead of 1000.0 cc. of water, neutralized by the addition of $CaCO_3$. Instead of adding 1.5 g. NH_4NO_3, 0.025 g. nitrogen was added in the form of NH_4NO_3 to each 100.0 cc. of solution. One of the following (1.0%) was added: sucrose, mannitol, galactose, lactose, inulin, glucose, arabinose, levulose, glycerol, starch. The solution was added to sand as before.
(b) Omitted the $CaCl_2$, used 1.0 g. K_2HPO_4 instead of 1.5 g. K_2HPO_4, omitted the $FeCl_3$, used only 500.0 cc. of water instead of 1000.0 cc., made slightly alkaline by the addition of Na_2CO_3, and added 0.0353 g. of nitrogen in the form of NH_4NO_3 to each 100.0 cc. of solution. To each 100.0 cc., 0.8 g. of one of the following was added: oxalic acid, acetic acid, succinic acid, malic acid, tartaric acid, citric acid, hippuric acid, uric acid, humus acid and aspartic acid. The solution was added to sand as before.

Reference: Münter (1913 p. 377).

2518. Gowda's Ammonium Sulphate Sand Medium

Constituents:

1. Water (conductivity)	1100.0 cc.
2. $(NH_4)_2SO_4$	1.0 g.
3. K_2HPO_4	1.0 g.
4. NaCl	2.0 g.

5. $MgSO_4$	0.5 g.
6. $Fe_2(SO_4)_3$	trace
7. $MgCO_3$	5.0 g.
8. Sand	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1000.0 cc. conductivity water.
(2) Dissolve 7 in 100.0 cc. conductivity water.
(3) Mix (1) and (2).
(4) Wash sand (Method not given).
(5) Mix solution (3) with sand (amount not given) and place in Petri dishes.

Sterilization: Method not given.

Use: To study nitrification by nitrate and nitrite forms. The author reported that nitrites were found in 10 days and nitrate after 14 days.

Reference: Gowda (1924 p. 259).

2519. Wichers' Ammonium Sulphate Sand Medium

Constituents:

1. Mineral Solution (nitrogen free).
2. Sand.
3. $(NH_4)_2SO_4$.
4. $MgCO_3$.

Preparation:

(1) Distribute nitrogen free mineral solution (composition not given) into 200.0 cc. Erlenmeyer flasks in 50.0 cc. lots.
(2) Add 24.0 g. sand to each flask.
(3) Add 10.0 cc. of a sterile 1.0% $(NH_4)_2SO_4$ solution and a knife point of sterile $MgCO_3$ to each sterile flask of (2).

Sterilization: Sterilize (2) in the autoclave. Method of sterilization of $(NH_4)_2SO_4$ solution not given.

Use: To study nitrification by nitrifying organisms. The author reported that nitrification was furthered in the presence of permutite. More permutite may be used.

Reference: Wichers (1920 p. 1).

2520. Malenkovic's Basal Nitrate Sand Medium

Constituents:

1. Water	100.0 cc.
2. KNO_3	0.15 g.
3. $(NH_4)_2HPO_4$	0.15 g.
4. K_2HPO_4	0.1 g.
5. $MgSO_4$	0.05 g.
6. Sand	

Preparation:

(1) Dissolve 2, 3, 4 and 5 in 1.
(2) Prepare 10.0% solutions of one of the soluble added nutrients in (1) and saturate pure Quartz sand (sea sand) with the solution. To those added nutrients that are solid, add (1) until they are saturated.

Sterilization: Not specified.

Use: Cultivation of *Coniophora cerebella* (*Corticium putaneum*). The author reported that all materials from which dextrose is derived were good sources of carbon.

Added nutrients: The author used one of the following materials:

glucose
levulose
d-mannose
d-galactose
maltose
sucrose
lactose
dextrin
starch
mannitol
dulcitol
xylan
arabinose
tannic acid
peptone
rhamnose
hog fat

The author saturated one of the following with the basic solution:

filter paper
cotton
cotton (soluble in KOH)
sulfite pulp (unbleached)
cellulose from pine
cellulose from beechwood

Reference: Malenkovic (1906 p. 412).

2521. Stigell's Nitrate Sand Medium

Constituents:

1. Water	992.5 cc.
2. $Ca(NO_3)_2$	4.0 g.
3. KNO_3	1.0 g.
4. $MgSO_4 + 7H_2O$	1.0 g.
5. K_2HPO_4	1.0 g.
6. Fe_2Cl_6	
7. Quartz sand	

Preparation:

(1) Dissolve 2, 3, 4, 5 and 6 in 1.
(2) Pour (1) over heated quartz sand (6 liters of (1) on 60 kgm. of sand).

Sterilization: Not specified.

Use: To study influence of bacteria on plants. The sand was inoculated with bacteria and seeds were planted. The following bacteria were used *Bacillus megatherium*, *Bacillus mesentericus fuscus*, *Proteus vulgaris*, *Bacillus pyocyaneus*, *Bacillus subtilis*, *Bact. coli commune*, *Bacillus prodigiosus*, *Bacillus butyricus*. The author reported that bacteria had little influence on germination ability.

Reference: Stigell (1909 p. 733).

2522. Krüger and Schneidewind's Basal Glucose Sand Medium

Heat sand to glowing, boil with HCl, heat to glowing and then saturate with medium 158.

Reference: Heinze (1906 p. 647).

2523. Ashby's Basal Mannitol Sand Medium (Reed and Williams)

Constituents:

1. Distilled water	1000.0 cc.
2. Mannitol	12.0 g.
3. KH_2PO_4	0.2 g.
4. $MgSO_4$	0.2 g.
5. NaCl	0.2 g.
6. $CaSO_4$	0.1 g.
7. $CaCO_3$	5.0 g.
8. Sand	

Preparation:

(1) Wash pure sea sand and burn.
(2) Distribute 15.0 g. of (1) in liter Erlenmeyer flasks.
(3) Dissolve 2, 3, 4, 5, 6 and 7 in 1.
(4) Add 100.0 cc. of (3) to each flask of (2) containing sand.
(5) Add from 250 to 2000 parts per million of one of the sterile added nutrients to each flask.

Sterilization: Sterilize (4) at 15 pounds pressure for 15 minutes. Method of sterilization of the added nutrients not given.

Use: To study nitrogen fixation. The authors reported that the fixation of nitrogen by Azotobacter was only slightly influenced by any of the compounds listed.

Added nutrients: The author added one of the following:

esculin
vanillin
daphnetin
cumarin
pyrocatechin
heliotropin
arbutin
resorcin
pyrogallol
phloroglucin
hydroquinone
salicylic aldehyde
oxalic acid
quinic acid
di-hydroxystearic acid
rhamnose
borneol
caffeine
betaine hydrochloride
trimethylamine
legumin
alloxan
cinnamic acid
asparaginic acid
hippuric acid
creatine
creatinine
xanthine
hypoxanthine
urea
formamide
glycocoll
allantoin
guanidine carbonate
nicotine
picoline
skatol
piperidine hydrochloride

Reference: Reed and Williams (1915 p. 168).

2524. Munter's Basal Carbohydrate Asparagin Sand Medium

Constituents:

1. Water	1000.0 cc.
2. $MgSO_4$	0.5 g.
3. NaCl	0.5 g.
4. K_2HPO_4	1.5 g.
5. $CaCl_2$	0.1 g.
6. $FeCl_3$	few drops
7. Glucose	10.0 g.

8. Glycerol	5.0 g.
9. Sand	

Preparation:

(1) Dissolve 2, 3, 4, 5, 6, 7 and 8 in 1.
(2) Distribute in 12.0 cc. lots.
(3) Add 12.0 cc. of (2) and 0.1 g. of one of the added nutrients to 50.0 g. sand.

Sterilization: Method not given.

Use: Cultivation of *Actinomyces odorifer*, *Act. chromogenes*, *Act. albus I* and *II*, *Act. S. a*, *b* and *c*. The author reported that alanin and tyrosin were the best nitrogen sources for the actinomycetes studied. The remainder of the compounds, however, were good sources for some of the actinomycetes.

Added nutrients: The author added one of the following: urea, sulphocarbamide alanin, tyrosin, dicyandiamide.

Variants: The author substituted 2.0 g. mannitol for 5.0 g. glycerol, used 2.0 g. K_2HPO_4 instead of 1.5 g. K_2HPO_4 in the basal solution. Then he used 0.02 or 0.1 g. of nitrogen in the form of NH_4NO_3, NH_4Cl or asparagin for added nutrients.

Reference: Münter (1913 p. 368, 377).

2525. Heinze's Beerwort Sand Medium

Constituents:

1. Beerwort.
2. Sand.

Preparation:

(1) Heat sand to glowing.
(2) Boil with HCl.
(3) Reheat to glowing.
(4) Saturate (3) with beerwort.

Sterilization: Not specified.

Use: To study the nitrogen assimilation of green algae, chlorella, chlorothecium stichococcus. Heinze reported that nitrogen was not assimilated.

Reference: Heinze (1906 p. 647).

2526. Krüger and Schneidewind's Extract Broth Sand Medium (Heinze)

Constituents:

1. Water	1000.0 cc.
2. Meat extract (0.5%)	5.0 g.
3. Peptone (0.5%)	5.0 g.
4. Glucose (0.5%)	5.0 g.
5. Sand	

Preparation:

(1) Dissolve 2, 3 and 4 in 1.
(2) Heat sand to glowing.
(3) Boil (2) with HCl.
(4) Reheat to glowing.
(5) Saturate (4) with (1).

Sterilization: Not specified.

Use: To study the nitrogen assimilation by green algae, chlorella, chlorothecium stichococcus. Heinze reported that nitrogen was not assimilated.

Reference: Heinze (1906 p. 647).

2527. Stigell's Bouillon Sand Medium

Constituents:

1. Water	97.0 cc.
2. Bouillon	3.0 cc.
3. Quartz sand	300.0 g.

Preparation:

(1) Prepare bouillon.
(2) Mix 300.0 g. of quartz sand, 3.0 cc. (1) and 97.0 cc. of water.
(3) Place in Petri dishes.

Sterilization: Method not given.

Use: To study influence of bacteria on rate of evaporation using *Bac. subtilis*, *Proteus vulgaris*, *Bac. coli communis*, and *Bac. mesentericus fuscus*. The author reported that evaporation was inhibited by the growth of bacteria. Amount of evaporation determined by loss of weight of cultures.

Reference: Stigell (1908 p. 60).

2528. Heinze's Loam Sand Medium

Constituents:

1. Distilled water.
2. Sand.
3. Loess loam.

Preparation:

(1) Add 35.0% sand to soft Loess loam soil rich in humus.
(2) Saturate with sterile distilled water.

Sterilization: Not specified.

Use: To study nitrogen assimilation by green algae, chlorella, chlorothecium, stichococcus. Heinze reported that nitrogen was not assimilated.

Reference: Heinze (1906 p. 647).

2529. Mortensen's "Starre" Media

Constituents:

1. Water	1000.0 cc.
2. Sucrose (10.0%)	100.0 g.
3. $(NH_4)_2SO_4$ (0.5%)	5.0 g.
4. Cobalt salts	
5. "Starre" materials	

Preparation:

(1) Dissolve 2, 3 and 4 in 1.

(2) Add (1) to one of the listed pulverized materials. The solid materials are placed in a Petri dish, and the solution added until they are about one-half immersed. The following "Starre" materials were used:

Quartz sand (Merck's sea sand, washed and heated to glowing).
Glass powder (pulverized Erlenmeyer flasks).
Kavlin (Merck)
Clay powder (pulverized pottery)
Clay plates (pieces of pottery)
Pieces of porcelain
Pumice stone powder
Kieselguhr
Talcum
Asbestos powder
Marble powder
Marble
Prepared chalk
$CaCO_3$ (precipitated Merck's)
Magnesium gypsum plates (1 part $MgCO_3$ + 99 parts gypsum, according to Omelianski).
Blood charcoal (Merck).
Charcoal powder.
Soot.
Pieces of sponge.

Sterilization: Not specified.

Use: See medium 329.

Variants: The author used media 329 or 560 instead of the solution given above.

Reference: Mortensen (1909 p. 532).

2530. Wojtkiewicz's Soil Medium

Constituents:

1. Water.
2. Soil.

Preparation:

(1) Fill 7.0 cm. wide and 20.0 cm. high glass cylinders with 500.0 g. of sifted soil that has been moistened with 25.0% water.

(2) Pass 10 liters of CO_2 free air thru the cylinder per day.

Sterilization: Not specified.

Use: To study carbon dioxide production by soil forms. The author collected the formed CO_2 in a Liebig's potassium apparatus. Sherman studied the activities of protozoa in the soil and their influence upon bacterial flora.

Variants:

(a) Sherman sterilized soil with a moisture content of 17.0% or 18.0%.

(b) Sherman prepared soil as follows:

(1) Place various soils in suitable containers.

(2) Cover with layer of non-absorbent cotton between layers of cheese cloth.

(3) Sterilize in autoclave at 15 pounds pressure for one hour.

References: Wojtkiewicz (1914 p. 260), Sherman (1916 pp. 45, 47).

2531. Makrinoff's Carbonate Soil Medium

Constituents:

1. Nitrite forming solution.
2. $MgCO_3$.
3. Soil.

Preparation:

(1) To each 10.0 g. of dry $MgCO_3$ add 0.25, 0.75, 1.0, 1.75, 2.5, 3.5 5.0 or 6.5 g. of dry soil.

(2) Mix well.

(3) Add usual substrata for nitrite formation (composition not given) until a semi fluid mass is formed.

(4) Place in test tubes and slant.

(5) Place a strip of absorbing paper in the tube touching the mixture and allow to stand over night.

(6) Solidification takes place.

(7) To each tube of sterile (6) add 3.0 to 5.0 cc. sterile substrata for nitrite formation.

Sterilization: Sterilize (6) for 30 minutes at 120°C. Method of sterilization of nitrite forming solution not given.

Use: To study nitrite production. The author reported that following inoculation growth took place, causing the $MgCO_3$ to become liquid. This caused the colonies to be deep in the substrata.

Reference: Makrinoff (1909 p. 417).

2532. Wichers' Ammonium Sulphate Permutite Medium

Same as medium 2519 but use 10.0 g. of calcium permutite instead of 24.0 g. of sand in each flask.

2533. Rössler's Iron Sulphate Brick Medium

Constituents:
1. Water.
2. Iron vitrol (hydrous ferrous sulphate).
3. Brick.

Preparation:
(1) Prepare a solution of iron vitrol in water.
(2) Place a sterile brick bat in (1).

Sterilization: Sterilize the brick bat by heating to glowing. Method of sterilization of (1) not given.

Use: Cultivation of *Crenothrix polypora.*

Reference: Rössler (1895 p. 189).

2534. Mortensen's Basal Nitrate "Starre" Medium

Same as medium 329, but the solution is added to one of the listed pulverized materials. The solid materials are placed in a Petri dish, and the solution added until they are about one-half immersed. The materials used were the same as in medium 2529.

2535. Greaves' Mannitol Soil Medium

Constituents:
1. Distilled water.
2. Soil.
3. Mannitol.

Preparation:
(1) Sterilize tumblers covered with Petri dishes.
(2) Add 100.0 g. of soil and 1.5 g. mannitol.
(3) Mix thoroly with a sterile spatula.
(4) Add enough sterile distilled water to make the moisture content about 18.0%.

Sterilization: Methods not given.

Use: To study nitrogen assimilation by soil forms. Nitrogen was determined by Lipman and Sharp's (Centr. f. Bakt., II Abt., 35: 648, 1913) modified Kjeldahl's method. The author reported that this medium was used to determine the nitrogen assimilation ability of different soils and 100.0 g. of the soils in question were used in (2).

Reference: Greaves (1914 p. 454).

2536. Vierling's Cellulose Soil Medium

Constituents:
1. Soil.
2. Filter paper.

Preparation: (1) Place a piece of filter paper between garden soil in a Petri dish.

Sterilization: Method of sterilization of (1) not given.

Use: Decomposition of cellulose. After inoculation place the medium under a glass bell jar. The author reported that filter paper showed no signs of being attacked. Growth occurred, however.

Reference: Vierling (1920 p. 206).

2537. Mortensen's Ammonium Sulphate "Starre" Medium

Constituents:

1. Water	1000.0 cc.
2. Sucrose (10.0%)	100.0 g.
3. $(NH_4)_2SO_4$ (0.5%)	5.0 g.
4. Cobalt salts	
5. "Starre" materials	

Preparation: Same as for medium 2534, but using a solution of 2, 3 and 4 in 1 instead of the basal solution.

Sterilization: Not specified.

Use: See medium 329.

Reference: Mortensen (1909 p. 532).

2538. Zikes' Glucose Peptone Soil Medium

Constituents:
1. Water.
2. Soil.
3. Glucose.
4. Peptone.

Preparation:
(1) Place soil in Erlenmeyer flasks to a depth of 1.0 cm.
(2) Pour water on one side and a 1.0% solution of glucose and peptone in the other side of the flask until the soil is dampened.

Sterilization: Sterilize (Method not given).

Use: Cultivation of *Apiculatus* yeast, *Torula alba, Torula Molischiana Mycoderma cerevisiae, Blastoderma salmonicolor.* Author reported that due to small glucose content the yeast produced spores on this medium.

Reference: Zikes (1911 p. 147).

2539. Mortensen's Peptone "Starre" Medium

Same as medium 560, but containing "starre" materials as given in medium 2529.

2540. Lipman's Dried Blood Soil Medium

Constituents:

1. Distilled water	18.0 cc.
2. Soil	100.0 g.
3. Dried blood	2.0 g.
4. NaCl	0.0 to 2.0%

Preparation:

(1) Add 2.0 g. of dried blood to each of a series of glass tumblers containing 100.0 g. of soil.

(2) To each tumbler add varying amounts of NaCl, increasing the salt concentration by 0.2% each time.

(3) Mix thoroly with a sterile dry spatula.

(4) Add 18.0 g. of sterile distilled water to each flask and mix thoroly.

(5) Cover the tumblers with clean Petri dish covers.

Sterilization: Not specified.

Use: To study ammonification by soil bacteria. The author reported that ammonification was inhibited by various amounts of NaCl, Na_2SO_4 and Na_2CO_3.

Variants: The author used Na_2SO_4, or Na_2CO_3 instead of NaCl.

Reference: Lipman (1911–12 p. 58).

2541. Greaves' Dried Blood Soil Medium

Constituents:

1. Water.
2. Soil.
3. Dried blood.

Preparation:

(1) Sterilize tumblers covered with Petri dishes.

(2) Weigh 100.0 g. of soil and 2.0 g. dried blood into each tumbler.

(3) Mix thoroly with a sterile spatula.

(4) Add sterile distilled water to make the moisture content about 18.0%.

Sterilization: Method not given.

Use: To study denitrification.

Reference: Greaves (1914 p. 449).

2542. Schönbrunn's Horn Meal Soil Medium

Constituents:

1. Distilled water	600.0 cc.
2. Soil	3000.0 g.
3. Horn meal	50.0 g.

Preparation:

(1) Mix thoroly 3000.0 g. soil with 50.0 g. horn meal.

(2) Place in glass beakers 25.0 cm. high and 15 cm. wide.

(3) Add 600.0 cc. distilled water.

(4) Cover the beakers with parchment paper.

Sterilization: Not specified.

Use: To study nitrification.

Reference: Schönbrunn (1922 p. 549).

2543. Löhnis and Green's Phosphate Meal Medium

Constituents:

1. Water (tap)	1000.0 cc.
2. K_2HPO_4 (0.05%)	0.5 g.
3. Soil	
4. Flesh meal	

Preparation:

(1) Dissolve 0.05% K_2HPO_4 in tap water.

(2) Mix 0.4 or 2.0 g. flesh meal with 50.0 cc. of (1).

(3) Add (2) to 100.0 g. soil (dry weight).

Sterilization: Not specified.

Use: To study ammonification by soil forms.

Variants: The authors used 0.2 or 1.0 g. blood or horn meal instead of flesh meal.

Reference: Löhnis and Green (1913 p. 536).

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Harvey's Oxalated Blood Agar, 1897.
Harvey's Pepsin Digest Serum Agar, 2042.
Harvey's Pepsinized Blood Agar, 2044.
Harvey's Peptic Blood Digest Agar, 1894.
Harvey's Phenol Peptone Agar, 1578.
Harvey's Placenta Blood Serum Agar, 1915.
Harvey's Purified Agar, 1401.
Harvey's Salicylate Infusion Agar, 1741.
Harvey's Saponin Blood Agar, 1885.
Harvey's Soil Infusion Medium, 1389.
Harvey's Sperm Egg Medium, 2444.
Harvey's Starch Agar, 1733.
Harvey's Starch Beef Infusion, 1333.
Harvey's Starch Infusion Broth, 850.
Harvey's Sucrose Egg Agar, 1871.
Harvey's Tartrate Peptone Solution, 646.
Harvey's Telluric Acid Peptone Solution, 533.
Harvey's Telluric Acid Serum Agar, 1923.
Harvey's Trypsinized Blood Agar, 1909.
Harvey's Trypsinized Caseinogen Blood Agar, 2056.
Harvey's Trypsinized Heart Egg Medium, 2449.
Harvey's Trypsinized Heart Solution, 1117.
Harvey's Trypsinized Meat and Kidney Solution, 1121.
Harvey's Trypsinized Ox Heart Agar, 2061.
Harvey's Tryptic Digest Solution, 1135.
Harvey's Tryptophane Peptone Solution, 676.
Harvey's Wheat Flour Solution, 1207.
Harvey's Whey Infusion Agar, 1944.
Harvey's Yeast Extract Agar, 2080.
Harvey and Iyengar's Dehydrated Broth, 778.
Harvey and Iyengar's Desiccated Bile Salt Trypsinized Mutton Agar, 2064.
Harvey and Iyengar's Desiccated Trypsinized Mutton Bouillon Agar, 2063.
Haslam's Brain Liver Infusion Broth, 834.
Hasting's Milk Agar, 2022.
Hata's Serum Tissue Medium, 1366.
Hauman's Pectin Peptone Solution, 655.
Havens and Taylor's Kidney and Blood Infusion Broth, 864.
Heap and Cadness' Basal Peptone Solution, 531.
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Hefferan's Basal Asparagin Solution, 377.
Hefferan's Peptone Rice Flour Medium, 2392.
Hefferan's Peptone Starch Medium, 2388.
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Hegner and Beckner's Blood Agar Solution, 945.
Heider's Wheat Agar (Klimmer), 2103.
Heim's Hemoglobin Agar, 1997.
Heim's Meat Extract Salt Agar, 1694.
Heiman's Pleuritic Serum Agar, 1933.
Heinemann's Ammonium Sulphate Agar, 1420.
Heinemann's Asparagin Peptone Agar, 1593.
Heinemann's Basal Sugar-Free Infusion Broth, 749.
Heinemann's Beer Wort Agar, 2088.
Heinemann's Beer Wort Gelatin, 2329.
Heinemann's Bread Paste Medium, 2508.
Heinemann's Bouillon, 812.
Heinemann's Glucose Agar, 1441.
Heinemann's Glucose Yeast Infusion Solution, 1142.
Heinemann's Glycerol Bouillon, 935.
Heinemann's Litmus Mannitol Agar, 1811.
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Heinemann's Meat Extract Agar, 1695.
Heinemann's Nitrate Broth, 814.
Heinemann's Peptone Extract Gelatin, 2298.
Heinemann's Sodium Nitrite Agar, 1427.
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Heinze's Beerwort Sand Medium, 2525.
Heinze's Glycogen Ammonium Sulphate Solution, 260.
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Heller's Indicator Nutrient Gelatin, 2309.
Heller's Peptone Urine Agar, 1658.
Heller's Starch Ammonium Sulphate Solution, 259.
Heller's Urine Peptone Gelatin, 2279.

Heller's Urine Peptone Solution, 745.
Henneberg's Asparagin Salt Solution, 419.
Henneberg's Basal Ammonium Sulphate Solution, 37.
Henneberg's Basal Asparagin Peptone Solution, 667.
Henneberg's Basal Glucose Di Sodium Phosphate Solution, 127.
Henneberg's Basal Glucose Salt Solution, 161.
Henneberg's Basal Nitrate Solution, 100.
Henneberg's Basal Sucrose Salt Solution, 135.
Henneberg's Basal Yeast Infusion Solution, 1141.
Henneberg's Glucose Extract Agar, 1754.
Henneberg's Glucose Extract Gelatin, 2303.
Henneberg's Glucose Peptone Salt Solution, 594.
Henneberg's Glucose Yeast Infusion Solution, 698.
Henneberg's Prune Infusion Peptone Solution, 711.
Henneberg's Sucrose Ammonium Acid Phosphate Solution, 218.
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Henssen's Glycerol Kidney Agar, 1738.
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Heraeus' Glucose Ammonium Carbonate Solution, 247.
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Hesse's Glycerol Agar, 1450.
Hesse's Glycerol Nährstoff-Heyden Agar, 1572.
Hesse's Lactose Glycerol Agar (Stokes and Hachtel), 1773.
Hesse's Malachite Green Agar (Klimmer), 1780.
Hesse's Starch Extract Agar (Stokes and Hachtel), 1763.
Hesse and Niedner's Glucose Peptone Agar, 1546.
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Heymann's Acetic Acid Glucose Bouillon (Finkelstein), 926.
Hibler's Blood Bouillon, 958.
Hida's Horse Meat Infusion Broth, 886.
Higgin's Basal Carbohydrate Asparagin Solution, 386.
Higgin's Basal Lactose Asparagin Agar, 1520.
Higgin's Glucose Nitrate Agar, 1469.
Higgin's Lactose Ammonium Succinate Gelatin, 2218.
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Hill's Artificial Milk Medium, 1317.
Hirsch and McKinney's Chocolate Agar, 1901.
Hirschbruch and Schwer's Azolitmin Crystal Violet Lactose Agar, 1795.
Hirschbruch and Schwer's Crystal Violet Litmus Lactose Agar, 1760.
Hiss' Basal Ascitic Fluid Peptone Solution (Park, Williams and Krumwiede), 729.
Hiss' Basal Asparagin Peptone Solution, 666.
Hiss' Basal Litmus Asparagin Solution, 391.
Hiss' Basal Litmus Milk Solution, 1294.
Hiss' Basal Serum Bouillon, 774.
Hiss' Basal Serum Peptone Medium, 727.
Hiss' Basal Serum Solution, 1253.
Hiss' Basal Urea Peptone Solution, 677.
Hiss' Extract Agar, 2185.
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Hitchen's Basal Sugar Free Agar Solution, 944.
Hitchen's Glucose Infusion Agar, 1723.
Hitchen's Glucose Agar Infusion Solution (Mulsow), 881.
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Hitchen's Yeast Autolysate Blood Solution, 878.
Hoffman's Nitrate Starch Agar, 1803.
Hoffman's Nitrite Starch Agar, 1804.
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Hogue's Ovomucoid Medium (Hegner and Becker), 1252.
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Hollborn's Meat Extract Gelatin, 2367.
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Holman's Basal Serum Extract Broth, 768.
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Holt, Harris and Teague's Eosine Methylene Blue Agar, 1778.
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Hucker and Wall's Glucose Peptone Agar, 1545.
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Hulton-Frankel's Inositol Infusion Agar, 1747.
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Hunter's Fish Infusion Broth, 798.
Hunter's Trypsinized Casein Extract Agar, 1851.
Huntoon's Hormone Agar, 1863.
Huntoon's Hormone Blood Agar, 1867.
Huntoon's Hormone Gelatin, 2292.
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Hurler's Caffeine Agar, 1816.
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Ickert's Yeast Extract Peptone Solution, 694.
Ickert's Yeast Infusion Peptone Agar, 1599.
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Jackson's Lactose Bile Solution, 1285.
Jackson and Melia's Bile Peptone Solution, 733.
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Jacobi's Infusion Broth Fucus Medium, 2381.
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Jacobi's Peptone Agar, 1530.
Jacobi's Peptone Fucus Medium, 2380.
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Jacobson's Ethylcinnamic Ether Agar, 1815.
Jacobson's Mannitol Extract Broth, 906.
Janke's Alcohol Ammonium Phosphate Solution, 297.
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Janke's Lagar Beer Agar, 2094.
Jenkins' Tomato Infusion Agar, 2112.
Jensen's Milk Digest Agar, 2047.
Jensen's Milk Digest Solution, 1112.
Jensen's Nitrate Infusion Broth, 780.
Jensen's Nitrate Soil Infusion Medium, 1394.
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Joas' Alkaline Serum Agar (Klimmer), 2004.
Jochmann's Potato Bouillon Agar, 1616.
Johnson's Glucose Phenol Extract Broth, 899.
Jones' Glucose Peptone Solution, 648.
Jones' Histidin-hydro-chloride Agar, 1525.
Jones' Modified Ashby's Mannitol Solution, 170.
Jordan's Basal Peptone Bile Agar, 1650.
Jordan's Phenol Infusion Broth, 857.
Jordan and Victorson's Lead Acetate Infusion Agar, 1676.
Jötton's Yeast Autolysate Agar, 2077.
Jötton's Yeast Autolysate Solution, 983.
Jouan's Citrate Lactose Bouillon (Besson), 932.
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Kabeshima's Alkaline Hemoglobin Agar, 1999.
Kabeshima's Hemoglobin Extract Agar, 2001.
Kahn's Casein Digest Ascitic Fluid Bouillon, 966.
Kahn's Casein Digest Egg Albumin Bouillon, 954.
Kammen's Pepsinized Yeast Agar (Klimmer), 2046.
Kammen's Yeast Digest (Klimmer), 978.

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Kappen's Cyanamide Asparagin Gelatin, 2227.
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Kendall, Day and Walker's Mannitol Bouillon, 936.
Kendall, Walker and Day's Ammonium Asparagin Solution, 412.
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Kent's Glycerol Albumin Solution, 1248.
Khouvine's Cellulose Agar, 1805.
Khouvine's Cellulose Peptone Solution, 626.
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Kiefer's Ascitic Fluid Agar (Abel), 1928.
Killer's Mannitol Solution, 179.
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Kindberg's Fuchsin Malachite Green Agar, 1798.
Kinsella, Brown and Garcia's Nutrose Gelatin Agar, 1878.
Kisch's Basal Glucose Agar, 1438.
Kisch's Basal Glucose Salt Solution, 129.
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Kita's Basal Ammonium Sulphate Solution, 39.
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Kitasato and Weyl's Chopped Beef Peptone Medium, 717.
Klecke's Basal Ammonium Phosphate Peptone Solution, 548.
Klecke's Basal Bouillon, 763.
Klecke's Lactose Ammonium Phosphate Solution, 230.
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Klein's Alkaline Serum Agar, 2013.
Klein's Basal Alkaline Serum Solution (Leuch), 1256.
Klein's Lactose Peptone Solution, 606.
Kligler's Blood Clot Infusion Broth, 797.
Kligler's Glucose Peptone Solution, 590.
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Kligler's Lead Acetate Glucose Agar, 1828.
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Kligler's Nasal Secretion Bouillon, 971.
Kligler's Nasal Secretion Serum Agar, 2009.
Kligler's Nitrate Peptone Solution, 561.
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Kligler's Yeast Autolysate Agar, 2075.
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Klimenka's Laked Blood Solution, 1271.
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Klinger's Malachite Green Extract Agar, 1708.
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Kodama's Fuchsin Sulphite Serum Agar, 2007.
Kodama and Takeda's Starch Peptone Solution, 625.
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Kohler's Basal Agar, 1709.
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Kohn's Peptone Extract Gelatin, 2300.
Kopp's Thyroid Agar, 2176.
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Korf's Sucrose Asparagin Solution, 433.
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Koser's Citrate Ammonium Phosphate Solution, 293.
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Krause's Gelatin Urea Agar, 1875.
Kreidler's Glucose Brain Broth, 862.
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Krumwiede and Pratt's Dahlia Agar, 1721.
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Krumwiede, Pratt and Grund's Egg Albumin Agar, 1954.
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Legroux's Formol Serum Solution, 1264.
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Lentz and Tietz's Malachite Green Bile Agar (Klimmer), 1934.
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Leuch's Basal Serum Solution (Klimmer), 1254.
Levine's Boric Acid Peptone Agar, 1547.
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Levine's Endo Agar, 1559.
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Loeffler's Malachite Green Nutrose Agar (Roddy), 1940.
Loeffler's Malachite Green Nutrose Peptone Agar, 1659.
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Löhnis' Basal Ammonium Chloride Solution, 33.
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Löhnis' Cyanamide Soil Infusion Gelatin, 2370.
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Löhnis' Glucose Peptone Solution, 591.
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Löhnis' Iron Ammonium Citrate Solution, 307.
Löhnis' Mannitol Soil Infusion Agar, 2197.
Löhnis' Pectin Ammonium Sulphate Solution, 263.
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Löhnis' Soil Infusion Medium, 1387.
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Lubinski's Glycerol Potato Agar, 2118.
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Lustig's Ammonium Chloride Peptone Solution, 649.
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Rosenow's Glucose Brain Agar (Haden), 1832.
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Sabouraud and Noire's Basal Whey Peptone Agar (Weil and Moire), 1654.
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Seliber's Basal Casein Peptone Solution, 743.
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Sherman's Basal Yeast Peptone Solution, 684.
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Sherman's Soil Hay Infusion Solution, 1180.
Sherman's Yeast Extract Peptone Agar, 1600.
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Sherwood and Downs' Basal Serum Agar, 1914.
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Shmamine's Liver Serum Bouillon, 963.
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Torrey and Buckell's Ascitic Fluid Egg Agar, 1868.
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